METABOLISM OF SOLUBLE AND STRUCTURAL CARBOHYDRATES DURING MUSKMELON FRUIT DEVELOPMENT

BY

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The objectives of this research were to 1) investigate changes in soluble sugar composition of muskmelon mesocarp during fruit development and to relate, if possible, the activity of invertase (E.C. 3.2.1.26) and sucrose synthase (E.C. 2.4.1.13), two enzymes of sucrose metabolism, to soluble sugar composition and 2) to determine if changes in structural polysaccharides were involved in muskmelon fruit softening.

Glucose and fructose in near equal amounts were the predominant sugars detected in muskmelon mesocarp tissue during the first 24 days after anthesis. Sucrose began to accumulate 24 days after anthesis and was the predominant sugar in the ripe fruit. A soluble acid invertase (pH optimum 4.0-5.0) was detected in mesocarp extracts. Invertase activity was highest in the young fruit and declined with development. Sucrose synthase activity was

also detected in mesocarp extracts. The specific activity of sucrose synthase was much lower than that of acid invertase. Sucrose synthase activity increased between 18 and 24 days after anthesis and then remained constant. Invertase apparently keeps sucrose from accumulating during the early stages of fruit development. Later in fruit development both invertase and sucrose synthase may have been responsible for the breakdown of sucrose.

Changes in cell wall components accompanied muskmelon softening. Total neutral sugar content of ethanol-insoluble powders decreased as ripening proceeded. Glucose and galactose were the predominant neutral sugars in ethanol-insoluble powders and were the sugars that decreased the most during softening.

Total polyuronide content decreased only slightly during softening; however, there was a significant increase in the quantity of soluble pectins. Gel chromatography of polyuronides revealed a decrease in large molecular weight polymers and an increase in small polymers with ripening. These results indicated that pectin degradation is involved in muskmelon fruit softening. However, polygalacturonase (E.C. 3.2.1.15) activity was not detected in muskmelon mesocarp.

A decrease in large hemicellulose polymers and an increase in small polymers also took place during softening. Neutral sugar components of hemicelluloses included arabinose, xylose, galactose, and glucose. On a mole

percent basis there was a decrease in galactose (51%) and glucose (37%) in the large polymers as fruit softened.

CHAPTER I
INTRODUCTION

Muskmelon fruit are valued for their sweet succulent flesh. The two principal components of muskmelon fruit quality are soluble sugar content and flesh texture. Soluble sugar content has long been used as an index of muskmelon maturity and quality (Rosa 1928, Currence and Larson 1941), and grade standards for muskmelons have been established based on degrees Brix of muskmelon juice (Pratt 1971). Several reports (Aulenbach and Worthington 1974, Yamaguchi et al. 1977) have indicated that soluble sugar content alone is not a reliable index of muskmelon quality; however, it is generally accepted that there is a positive correlation between soluble sugar content and quality, and that melons with low soluble sugar content will never be considered high quality. Flesh texture is not only a component of eating quality (Rosa 1928, Gilbart and Dedolph 1963, Olge and Christopher 1957, Yamaguchi et al. 1977, Evenson 1983) but it is also related to the postharvest longevity of the crop (Olge and Christopher 1957).

Although numerous studies have been conducted concerning changes in soluble sugar content and flesh texture during muskmelon fruit development and in response

to various pre- and postharvest treatments, information regarding the physiological basis of these changes is lacking. Accumulation of sugar by fruit tissues in general is a rather poorly understood phenomenon (Willenbrink 1982) and what little is known indicates that there is variation among various fruit types. Considerably more research has been conducted on the changes in flesh texture that take place during fruit ripening; however, few studies have dealt with muskmelons even though the fruit undergoes considerable softening.

The objective of the work described in this dissertation was 2-fold. One aspect involved an investigation of the changes in soluble carbohydrate composition during muskmelon fruit development and the activity of invertase and sucrose synthase, two enzymes of sucrose metabolism. The second aspect involved a characterization of cell wall changes related to the fruit softening process.

CHAPTER II
LITERATURE REVIEW

Muskmelon Fruit

Taxonomy and Fruit Morphology

Muskmelon is a member of the Cucurbitaceae (Whitaker and Davis 1962) and is the common name for botanical varieties and cultivars of *Cucumis melo* L. (Whitaker 1970). Fruit of *Cucumis melo* L. var. *reticulatus* Naud. are commonly referred to as "cantaloupes" but as pointed out by Whitaker (1970) this is incorrect; therefore, the term muskmelon will be used in this text. Fruits of this species show a considerable degree of morphological variation. They vary in size, shape, and external appearance. They may be smooth or have vein tracts (Davis 1970), they may be netted or partially netted, the rind color at maturity varies from yellow to yellow-brown or greenish yellow (Whitaker and Davis 1962), and the flesh color may vary from green to salmon orange (Whitaker 1970).

Muskmelon fruit develop from perfect flowers with inferior ovaries and are classified as modified berries or pepos (Webster 1975). The ovaries are tricarpellate and usually contain somewhat greater than 100 ovules (Mann and Robinson 1950). The fruit is composed of the pericarp and

adenate floral tube; a leathery exocarp and fleshy mesocarp are the two most prominent tissues of the pericarp (Whitaker and Davis 1962). The mesocarp is composed of large thin-walled isodiametric parenchymatous cells; bundles and phloem elements are found throughout the mesocarp.

Fruit Growth and Development

Fruit set in muskmelon is dependent on adequate pollination; if pollination is not sufficient, ovaries will shrivel and abort within several days after anthesis (Mann and Robinson 1950). If pollination is adequate, fruit set is achieved and the ovaries begin to enlarge rapidly.

McGlasson and Pratt (1963) investigated fruit growth in 'PMR 45' muskmelons and reported that the fruit exhibit a simple sigmoidal growth curve that could be divided into three phases. The first phase includes anthesis and ends approximately ten days after anthesis; during this phase there is an exponential increase in ovary volume. The second phase is represented by a constant growth rate. The third phase of growth is represented by a slower, but constant growth rate which continues until the fruit abscises. The same general pattern of fruit growth has been reported for a number of C. melo genotypes (Pratt et al. 1977, Bianco and Pratt 1977); however, as pointed out by Pratt (1971) considerable differences exist among

genotypes in the absolute size of the fruit and number of days from anthesis to abscission.

Muskmelon fruits generally abscise from the plant 35-55 days after anthesis depending on the cultivar and environmental conditions (Pratt 1971, Kasmire 1981). The timing of muskmelon harvest is determined by the development of the abscission layer at the base of the peduncle. Depending on the extent of separation between the fruit and the peduncle, the fruit is described as being in the "forced slip," "half slip," or "full slip" stage (Rosa 1928, Webster 1975). "Full slip" is the time of complete abscission.

Fruit Ripening

Lyons et al. (1962) investigated respiration and ethylene evolution in muskmelon fruits at various stages of development and reported a climacteric rise in respiration during ripening. A marked increase in ethylene production coincided with or immediately preceded the respiratory rise. The preclimacteric minimum coincided with the half slip stage and climacteric peak with the full slip stage in the melon cultivar studied. McGlasson and Pratt (1964) found that treatment of mature but preripe (25 days after anthesis) muskmelon fruit with 0.1 ppm ethylene decreased the length of time required to reach the climacteric peak and enhanced the uniformity of ripening. Increasing the concentration of ethylene from 0.1 to 1.0 or 10.0 ppm

further enhanced uniformity of ripening. Although all preclimacteric muskmelon fruit had an increase in respiration due to ethylene treatment, only fruits older than 20 days exhibited a true respiratory climacteric and the other changes associated with normal ripening. This response indicates that prior to 20 days after anthesis the fruits are physiologically immature.

Rowan et al. (1969) found that mesocarp ATP content doubled during the climacteric rise in muskmelons, while ADP content remained constant, indicating a net synthesis of adenosine pyrophosphates during ripening. Ethylene treatment stimulated a climacteric rise in fruit between 9 and 32 days postanthesis; however, the ATP/ADP ratio increased only in fruit 20 or more days after anthesis.

Changes which occur during ripening in muskmelon fruit include a rapid accumulation of soluble sugars (Rosa 1928, Bianco and Pratt 1977, Pratt et al. 1977, Hughes and Yamaguchi 1983, Lester and Dunlap 1985), loss of flesh firmness (Bianco and Pratt 1977, Bianco et al. 1977, Lester and Dunlap 1985), loss of chlorophyll (Reid et al. 1970), and the production of volatile compounds.

Accumulation of Soluble Sugars

Introduction

Fruits are strong physiological sinks for phloem-translocated carbohydrates (Bollard 1970, Coombe 1976).

The strong sink strength of developing fruits is indicated by the large percentage of dry weight allocated to them (Hansen 1970, Hall 1977, Fourney and Breen 1985a). The predominant form in which carbohydrates accumulate in fruit flesh is soluble sugars; however, considerable variation exists in the carbohydrate composition of various fruit types (Widdoson and McCance 1935, Coombe 1976).

The changes in soluble sugar content and composition during muskmelon fruit development have received considerable attention owing to the importance of soluble sugars to quality. Rosa's (1928) early findings have been confirmed in a number of more recent reports (Bianco and Pratt 1977, Pratt et al. 1977, Hughes and Yamaguchi 1983, Lester and Dunlap 1985). During the early stages of fruit development total soluble sugar content is low and is composed of glucose and fructose which are present in nearly equal concentration. At the onset of ripening total soluble sugar content begins to increase rapidly, due to an increase in sucrose; glucose and fructose contents remain fairly constant or may decrease slightly. According to Bianco and Pratt (1977) greater than 97% of total soluble solids in muskmelon fruit is in the form of soluble sugar, and sucrose accounts for the greatest percentage of the total soluble sugar in mature muskmelon fruit. A similar pattern of soluble sugar accumulation occurs in developing

watermelon fruit (Elmstrom and Davis 1981, Brown and Summers 1985).

Mechanism of Carbohydrate Accumulation

Although our understanding of the mechanisms which regulate phloem unloading and subsequent accumulation in sink tissues, and especially fleshy fruits, is rather limited, some generalizations can be made. First, unloading of carbohydrate from the phloem may be symplastic, apoplastic, or a combination of both depending on the particular sink tissue (Geiger and Foundy 1980, Giaquinta 1980, Ho and Baker 1982). Second, the accumulation of carbohydrate by sink tissues requires the expenditure of metabolic energy; however, the site(s) of energy input may vary depending on the particular sink (Giaquinta 1980). Third, sink strength is dependent on the ability of the sink tissue to deplete the concentration of translocate in the phloem thereby maintaining a concentration gradient favorable for movement. This depletion may be accomplished by direct utilization in respiration, chemical transformation, spatial compartmentalization, or a combination of all three (Giaquinta 1980, Ho and Baker 1982). Obviously, for net accumulation of carbohydrate to occur the amount respired must be low in relation to the amount imported. Therefore transformation and spatial compartmentalization must be important mechanisms in the accumulation process.

Vacuole

It is generally accepted that soluble sugars accumulate in the vacuoles of fruit flesh (Coombe 1976); however, few studies have been conducted on isolated vacuoles. Yamaki (1984) isolated vacuoles from immature apple fruit flesh and compared their contents with the contents of protoplasts. The vacuoles contained essentially 100% of the fructose, glucose, and sorbitol, but only 10% of the sucrose. Of the total sugars, fructose, glucose, sucrose, and sorbitol represented 50%, 40%, 4%, and 3%, respectively. Coombe and Matile (1980) studied solute accumulation in grape berry skin segments and determined that glucose was compartmentalized in the vacuole. Apple fruit tissue disks accumulated sorbitol from the bathing solution in a manner that suggested involvement of a carrier-mediated system, i.e., saturation kinetics and inhibition by competing sugars (Beruter and Kalberer 1983). Based on these results it was suggested that sorbitol uptake is regulated by a carrier-mediated transport system on the tonoplast.

Sugar Transformation

One of the earliest changes detected following fruit set is an alteration in carbohydrate composition of the ovary. Within 48 hours after pollination of tomatoes there was a marked increase in starch content, a slight increase in reducing sugar, and a decrease in sucrose in the ovaries

(Marre and Murneek 1953). In pepper and watermelon ovaries during the first nine days after pollination fruit growth was accompanied by significant increases in reducing sugar concentration (Walker and Hawker 1976). Thirty-six hours after tomato fruit were induced to set by treatment with napthoxyacetic acid there was a significant increase in the movement of ^{14}C-labeled-sucrose into the ovaries as compared with water treated controls (Archbold, Dennis and Flore 1982).

Walker and Ho (1977a) investigated the translocation of carbon into the tomato fruit in order to determine the relationship between the rate of carbon import and changes in the levels of major carbon metabolites. Sucrose accounted for 90% of the carbon imported into the fruit. However, in the fruit sucrose was rapidly hydrolyzed to hexoses which were utilized for respiration and for the synthesis of cell wall material, starch, and amino and organic acids. There was a positive correlation between the rate of sucrose import and the synthesis of starch and cell wall material. The absolute rate of carbon import was greater, and the rate of respiration lower in small fruit compared to large fruit. It was concluded that sink strength was more dependent on sink activity than on sink size.

In a subsequent study Walker and Ho (1977b) examined the relationship between carbohydrate metabolism of the

fruit and the rate of carbon import. By cooling or warming the fruit it was possible to alter the rate of sucrose import. At 5°C sucrose accumulated in the fruit; at 35°C sucrose accumulation was reduced. Sucrose import was inhibited by cooling and enhanced by warming. Synthesis of sucrose from hexoses was enhanced in fruit at 5°C (Walker and Ho 1977b). Based on these results it was suggested that sucrose concentration in the fruit regulates the rate of translocation of sucrose into the fruit.

In apple, sorbitol is the major sugar translocated in the phloem, but sorbitol does not accumulate in the fruit flesh (Hansen 1970, 1979). Beruter and Kalberer (1983) studied the uptake of sorbitol by apple fruit tissue disks. The rate of sorbitol uptake was correlated with the rate of conversion of sorbitol to fructose, the principal sugar present in the apple fruit.

In grapes, sucrose is the principal sugar translocated from leaves in the phloem (Swanson and El Shishiny 1958) but glucose and fructose are the sugars that accumulate in the berries (Kliewer 1966).

Although glucose, fructose, and sucrose are the sugars present in the flesh of melons as well as other cucurbit fruits (McCreight et al. 1978, Pharr et al. 1977, Handley et al. 1983) stachyose and raffinose constitute the principal sugars present in the translocation stream of cucurbits (Hendrix 1982, Hughes and Yamaguchi 1983,

Richardson et al. 1982, 1984, Webb and Burley 1964). Therefore, stachyose and raffinose must be converted to hexose prior to entering the fruit. Gross and Pharr (1982b) described a pathway for stachyose catabolism in cucurbit peduncles involving the sequential action of α-galactosidase, galactokinase, UDP-galactose pyrophosphorylase, UDP-galactose-4-epimerase, and sucrose synthase.

In cucumber, sucrose is the sugar translocated from the peduncles into the developing fruit and through the fruit to the funiculi (Handley et al. 1983). However, sucrose is present in very low quantities in the cucumber fruit. Presumably a similar pathway of sugar transport exists in muskmelon because stachyose and raffinose are present in the translocation stream but not in the fruit (Hughes and Yamaguchi 1983). Although the conversion of stachyose and raffinose to sucrose occurs in the peduncles of cucurbits, glucose, and fructose are the sugars present in young fruit; sucrose does not begin to accumulate until the latter stages of fruit development. No information concerning the sugar transformation in the flesh tissue of muskmelon is available.

Invertase

Invertase (β-fructofuranosidase, E.C. 3.2.1.26) catalyzes the hydrolysis of sucrose to glucose and fructose. The enzyme is found in a wide range of plant

tissues and has been the subject of numerous investigations. The literature on invertase in plants has been reviewed by several authors (ap Rees 1974, Avigad 1982, Hawker 1985). Based on differences in pH optima, two forms of the enzyme are recognized: acid invertase with a pH optimum in the range of 4.0-5.0, and neutral or alkaline invertase with a pH optimum in the range of 7.0-7.8. The acid form of the enzyme is more common than the alkaline form. Acid invertases have apparent K_m values for sucrose between 2 and 13 mM and alkaline invertases between 9 and 25 mM.

Considerable variation has been reported concerning the solubility of invertase; however, in many cases solubility may be related to the extraction conditions. Arnold (1965) reported that 25% of the total invertase in grape berries was insoluble. Insolubility was enhanced by the presence of fruit skins and seeds during homogenization. Hawker (1969b) found that the solubility of grape berry invertase was related to the formation of protein-tannin-cell wall complexes that formed during extraction. Treatment of the insoluble material with Carbowax or borate buffer solubilized the bound enzyme; inclusion of Carbowax in the extraction buffer rendered 95% of the total invertase soluble.

Nakagaw et al. (1971a) found that binding of invertase to tomato fruit cell wall was decreased by 20% in the

presence of 0.1 M Ca or Mg, suggesting that invertase binds to cell wall via ionic interaction. In a subsequent study (Nakagawa et al. 1971b), it was found that invertase was not released from tomato fruit cell wall at pH values less than 5.0.

Beruter (1985) investigated invertase activity in apple fruit, but made no mention of the solubility of the enzyme. Yamaki and Ishikawa (1986) reported that invertase activity was detected only in the insoluble fraction from apple fruit; no attempt was made to solubilize the enzyme.

No bound invertase was found in papaya fruit (Chan and Kwok 1976). In watermelon and pepper ovaries both bound and soluble invertases were detected; however, soluble invertase accounted for the greatest percentage of total activity (Walker and Hawker 1976).

In citrus fruit, acid invertase was found in the soluble fraction, alkaline invertase was present in both the soluble and insoluble fractions, and its solubility changed during fruit development (Kato and Kubota 1978). Alkaline invertase was least soluble in the immature fruit and solubility increased with fruit maturation.

Due to the effects of extraction conditions on the binding of invertase, cell fractionation is not a reliable indicator of invertase location (ap Rees 1974). However, pH optima indicate a vacuolar location for the acid form of the enzyme and a cytoplasmic location for the alkaline

form. The acid form of invertase in fruit tissue is often assumed to be vacuolar (Coombe 1976, Hawker 1985, Beruter and Kalberer 1983). Studies by Leigh et al. (1979) with vacuoles isolated from beet root showed that most of the invertase in that tissue was located in the vacuole.

In most studies there is a negative correlation between invertase activity and sucrose concentration. Developing grape berries accumulate glucose and fructose in 100-fold excess of sucrose (Kliwer 1966); invertase activity increases at the time of most rapid sugar accumulation in developing grape berries (Hawker, 1969a). Manning and Maw (1975) found higher invertase activity in fruits of Lycoperscicon pimpinellifolium than in L. peruvianum; sucrose concentration was lower in L. pimpinellifolium than in L. peruvianum. The low temperature induced accumulation of reducing sugars in grapefruit was correlated with an increase in invertase activity (Purvis and Rice 1983).

The role of invertase is assumed to be the catalysis of sucrose breakdown when there is a strong demand for hexose as in rapidly growing tissues (ap Rees 1974). In fruit tissues invertase activity is often greatest during early development, i.e., the time of most rapid growth. Watermelon and pepper ovaries increased 5-fold and 1.5-fold, respectively, in invertase activity in the first nine days following pollination (Walker and Hawker 1976).

Invertase activity in strawberry (Poovaiah and Veluthambi 1985) and apple (Beruter 1985, Yamaki and Ishikawa 1986) fruit was highest at anthesis and declined during development. In citrus, acid invertase activity was highest in immature fruit and it declined during development; alkaline invertase activity followed a similar trend, but activity did not decline to the same extent as did acid invertase (Kato and Kubota 1978).

In contrast with the previously described fruits, tomato shows an increase in invertase activity during development (Manning and Maw 1975, Iwatsubo et al. 1975). There was a 2.6-fold increase in the invertase activity from the mature green to the red stage and approximately a 1.5-fold increase from the red to overripe stage. This increase in invertase activity during ripening may be related to the increased demand for hexose required for respiration during the climacteric. In senescent tomato fruit (30 days after harvest at mature green stage) there was a decrease in invertase activity when compared to ripe fruit (Nakagawa et al. 1980).

Sucrose Synthase

Sucrose synthase (UDP glucose:D-fructose 2-glucosyl-transferase, E.C. 2.4.2.13) catalyzes the reaction:

$$\text{UDP-glucose} + \text{fructose} \rightleftharpoons \text{sucrose} + \text{UDP}.$$

The literature on sucrose synthase has been reviewed in several recent reviews (ap Rees 1974, Pontis 1977, Avigad

1982, Hawker 1985). In the following discussion, information not referenced specifically has been obtained from these reviews.

Sucrose synthase has been found in a wide variety of plant tissues and is presumably present in all tissues that are capable of metabolizing sucrose. Activity of the enzyme is highest in tissues that are synthesizing starch or cell wall material. Most evidence indicates that sucrose synthase functions *in vivo* in the direction of sucrose breakdown in order to provide sugar nucleotide precursors for starch and cell wall synthesis. However, Gross and Pharr (1982a) have demonstrated that there are two forms of sucrose synthase present in cucumber peduncles. Based on differences in pH optima and K_m for UDP-glucose it was suggested that one of the isozymes functions to synthesize rather than degrade sucrose in cucumber peduncles.

Only a limited number of studies have investigated sucrose synthase activity in developing fruits. Bean (1960) reported that cell-free preparations from citrus fruit are capable of synthesizing sucrose from fructose and UDP-glucose. Sucrose synthase has been detected in young watermelon and pepper fruits, but activity was much lower than invertase (Walker and Hawker 1976). In grape berries sucrose synthase activity decreased during the first six

weeks of fruit development, and then increased (Hawker 1969a).

Claussen (1983a) investigated sucrose synthase activity in eggplant and found that growing fruit had the highest activity of all organs and accounted for 90% of the total activity. In a subsequent study (Claussen 1983b) found a positive correlation between fruit growth rate and sucrose synthase activity. In studies with detached leaves of eggplants, sucrose synthase activity was greatest in leaves which acted as sinks rather than leaves which acted as sources (Claussen et al. 1984). Based on these results, it was concluded that sucrose synthase was a good indicator of the sink strength of an organ.

Fruit Softening

Introduction

The loss of flesh firmness, or softening, that occurs during fruit ripening has important horticultural implications. Flesh texture is not only an important component of fruit quality, but is also related to postharvest longevity. As fruits soften they become more susceptible to mechanical damage and invasion by pathogens.

The edible portion of fleshy fruits is composed of large, thin-walled, isodiametric parenchymatous cells (Coombe 1976, Knee and Bartley 1981). The cell wall provides structural integrity and regulates flesh

texture. It is generally accepted that fruit softening is a consequence of enzymic and perhaps nonenzymic mechanisms that alter cell wall structure (Huber 1983b).

Cell Wall Structure

The literature on the composition and structure of the primary cell walls of angiosperms has been reviewed by a number of authors (Albersheim 1976, 1978; Aspinall 1980, Darvill et al. 1980). From these reviews it is apparent that there exists a fundamental similarity between the primary cell walls of most tissues investigated.

It is well accepted that the primary cell walls of higher plants are composed of four basic types of macromolecules: cellulose, hemicellulose, pectic polysaccharides, and glycoproteins. Cellulose molecules form the fibrillar component of the cell wall and are embedded in an amorphous matrix of polysaccharides and glycoproteins.

Early definitions of cell wall polysaccharides were based on differential solubilities of the various cell wall components. Pectins were regarded as the fraction soluble in hot water, ammonium oxylate, weak acid, or chelating agents. Hemicelluloses as that component soluble in strong alkali. Cellulose represents the insoluble material that remains after pectins and hemicellulose have been removed. A more contemporary classification of cell wall

components is reasonably close to the early definitions. Pectins are those polymers soluble in weak acid covalently associated with galacturonosyl-containing polysaccharides. Hemicelluloses are soluble in alkali, hydrogen bonded to cellulose, and are composed of xyloglucans, xylans, and heteroxylans.

Pectins. Pectic polysaccharides may represent 35% of the primary cell wall material in dicots (Darvill et al. 1980). The most characteristic feature of these polymers is the high content of galacturonosyl residues. Rhamnogalacturonans form the backbone of the pectic polymers, but other covalently bound sugars are also present. Araban and galactan side chains are attached to the rhamnogalacturonan backbone. The area between the primary cell walls is known as the middle lamella and is particularly rich in pectic polysaccharides. The middle lamella is also the region most affected during fruit softening (Huber 1983b).

Hemicelluloses. Hemicelluloses of the primary cell wall of dicots are composed principally of xyloglucans, the components of which are glucose, xylose, galactose, and in some cases fucose. Hemicelluloses are believed to hydrogen bond to cellulose. The extent of covalent attachment between hemicellulose and pectins is unknown.

Cellulose. Cellulose represents the fibrillar component of the cell wall and is composed of chains of β-1,4 linked glucose.

Protein. The role of protein in cell wall structure remains an enigma. Attachment of hydroxy-proline-rich glycoprotein to other cell wall components has not been confirmed.

Cell Wall Changes Related to Softening

Microscopic examination of cells from softening fruit has revealed degradation of the cell wall material and an increase in separation of adjoining cells. Neal (1965) reported that during ripening of strawberry fruit there is extensive cell separation in the receptacle tissue. In apples, cell walls from mature, firm fruit were darkly stained and there was tightly packed fibrillar material in the walls of adjacent cells (Ben-Arie et al. 1979). Soft, mealy, overripe fruit showed destruction of the middle lamella. The most obvious change in the ultrastructure of mesocarp cells from ripening avocado fruit was a loosening and eventual breakdown of the cell wall; in postclimacteric fruit the cell wall had nearly disappeared (Platt-Aloia and Thomson 1981). Crookes and Grierson (1983) reported that the middle lamella in mature, preclimateric tomato fruit appeared as an electron dense region in transmission electron microscopy. Three to four days after the increase

in ethylene evolution, a time correlated with initial decrease in firmness, degradation of the middle lamella became apparent.

The degradation of cell wall material during softening is reflected in the reduction in yield of insoluble residue. Wallner and Bloom (1977) reported a 33-37% decrease in the cell wall weight of tomato fruit during ripening. In 'Bartlett' pears there was a loss of more than one gram of water-soluble carbohydrate per fruit during the transition from mature green (firm) to overripe (soft) (Ahmed and Labavitch 1980). In strawberry, a loss of ethanol-insoluble material occurs on a gram fresh weight basis; however, on a per fruit basis there is an increase in the yield of ethanol-insoluble residue (Huber 1984). The strawberry fruit is unique in that growth continues during the ripening process.

Compositional changes. An increase in the solubility of pectic polysaccharides has long been recognized as a general feature of the softening process in a wide range of fruit types (Pilnik and Voragen 1970, Pressey 1977). In muskmelon, total pectin content remains fairly constant during ripening (Rosa 1928, Lester and Dunlap 1985); however, there is a transition from insoluble protopectin to soluble pectin and pectic acid during ripening (Rosa 1928).

Pressey and Avants (1978) were able to show a very clear relationship between pectin solubility and fruit firmness in comparisons of several cultivars of clingstone and freestone peaches. Both types of peaches have low quantities of soluble pectins prior to ripening. During ripening, firmness decreased more rapidly in the freestone cultivars, accompanied by larger increases in the quantity of soluble pectins compared to the clingstone varieties.

During ripening of pears there is a significant increase in the quantity of water-soluble pectins (Yamachi et al. 1979, Ahmed and Labavitch 1980). Although the solubility of pectins increased during softening of 'Bartlett' pears, the most rapid losses of uronic acid did not occur until substantial tissue softening had occurred (Ahmed and Labavitch 1980). These authors concluded that the initial metabolism of cell wall pectins could lead to a decrease in firmness without altering pectin solubility.

Pectic polysaccharides comprised approximately 60% of the pericarp cell wall from mature green tomatoes and the loss of wall components during softening was restricted to this fraction (Gross and Wallner 1979). There was an increase in total pectin content of ethanol-insoluble powders prepared from both pericarp and locular gel of tomato fruit during ripening (Huber and Lee 1986). In ethanol-insoluble powders prepared from immature green fruit, soluble pectins represented approximately 24% of the

total pectins; during ripening the percent of soluble pectins increased and in powders prepared from ripe fruit, soluble pectins represented 60% of the total pectins.

Gross (1986) investigated the composition of ethanol-insoluble carbohydrates in water-soluble extracts of ripening tomatoes. There was a substantial increase (79%) in the quantity of ethanol-insoluble polysaccharides in water-soluble extracts as ripening proceeded. A 7.4-fold increase in the uronic acid content and a 4-fold increase in the rhamnose content of the ethanol-insoluble polysaccharide composition indicated an increase in the amount of water-soluble pectins.

Knee et al. (1977) reported that in strawberry fruit during the first 21 days after petal fall, greater than 90% of the total polyuronides were insoluble. In contrast, from 21 to 35 days after petal fall, nearly 70% of the total polyuronides in ripe berries were soluble. Total polyuronides as a percentage of ethanol-insoluble powders remained constant during strawberry fruit development, but the proportion of soluble polyuronides increased from 30% in undeveloped fruit to 65% in ripe fruit (Huber 1984).

In addition to the increase in the solubility of pectic polysaccharides, losses of cell wall neutral sugars have also been found to occur during fruit softening. Gross and Sams (1984) reported loss of neutral sugars during ripening in 15 of 17 fruit types. A net loss of

cell wall galactose and arabinose was detected in 14 of the species.

In strawberry fruits, the quantity of xylose, mannose, and glucose in the soluble fraction of cell-wall extracts increased during ripening indicating that hemicellulosic polymers were being degraded or released from interpolymeric bonds (Knee et al. 1977).

Knee (1973b) investigated cell wall changes in apples ripening either attached or detached from the tree. There was a significant decrease in cell wall glucan during ripening, although the decrease was more pronounced in detached fruit than in fruit attached to the tree.

A significant loss of arabinose and galactose occurs during ripening in pears (Yamaki et al. 1979, Ahmed and Labavitch 1980). However, Ahmed and Labavitch (1980) attributed the loss of arabinose during pear fruit softening to the solubilization of a pectic arabinan and therefore considered this to be a direct consequence of pectin solubilization. In tomato fruit there is a ripening-related loss of galactose and arabinose that occurs separately from polyuronide solubilization (Gross and Wallner 1979).

Hot pepper fruit have a relatively low (10%) neutral sugar content; however, the total amount of neutral sugar decreased more than 50% during ripening (Gross et al.

1986). The loss of neutral sugar was largely related to a decrease in galactose (76%) and arabinose (38%) content.

Changes in polymerization of wall polymers. From the literature reviewed it is apparent that fruit softening involves changes in cell wall composition which are well correlated with the loss of wall integrity. Huber (1983a) suggested that information gained from compositional analysis is somewhat limited because it reflects changes only in polymers that are lost from the wall and is not sensitive to modifications which may affect wall polymers without altering solubility or causing a net loss of their component sugars. Another technique which has been used to investigate cell wall modifications related to fruit softening is gel-filtration chromatography. Although this technique cannot be used to determine precisely molecular size of cell wall polymers, the elution profiles may be used to visualize changes in wall components during softening.

Gel-filtration profiles of polyuronides extracted from tomatoes revealed a decrease in the proportion of large molecular weight polymers and an increase in small polymers as ripening proceeded (Huber 1983a). These changes indicate that the increases in pectin solubility during softening are related to the degradation of pectins.

In strawberry fruit, although there was an increase in the solubility of pectins during ripening, there was no

corresponding shift in the elution profiles of pectins, indicating that the increase in pectin solubility was not the result of pectin degradation (Huber 1984). These results are in contrast with those reported by Woodward (1972) who found that the viscosity of soluble polyuronides extracted from ripening strawberries was lower than expected, indicating a breakdown of these polymers.

Hemicelluloses extracted from tomato pericarp showed a transition in elution pattern as ripening proceeded (Huber 1983a). Large molecular weight hemicellulloses were predominant in extracts from mature-green fruit; there was a decrease in the large molecular weight polymers and an increase in smaller polymers as the fruit ripened. Hemicelluloses extracted from locular gel have also been reported to shift from predominantly large polymers to smaller polymers as ripening proceeded (Huber and Lee 1986).

Gel-filtration profiles of hemicelluloses from ripening strawberry fruit showed significant changes during ripening (Huber 1984). Prior to the initiation of ripening there was a predominance of large molecular weight hemicelluloses. The initial change, a decrease in the proportion of large molecular weight polymers, became apparent at the onset of ripening. As ripening proceeded there was a continued decrease in large molecular weight

hemicelluloses and an increase in the small molecular weight hemicelluloses.

Gross et al. (1986) reported that the shift in elution profiles of hemicelluloses from ripening hot pepper fruit showed a transition from higher molecular weight polymers to lower molecular weight polymers as ripening progressed.

CHAPTER III
SOLUBLE SUGAR ACCUMULATION, INVERTASE, AND SUCROSE SYNTHASE ACTIVITY

Introduction

The changes in soluble sugar content and composition during muskmelon (Cucumis melo L. var. reticulatus Naud.) fruit development have received considerable attention owing to the importance of soluble sugars to muskmelon fruit quality. Rosa's (1928) early findings have been confirmed in a number of more recent reports (Bianco and Pratt 1977, Hughes and Yamaguchi 1983, Lester and Dulap 1985, Pratt et al. 1977). During the early stages of fruit development total soluble sugar content is low and is composed of glucose and fructose which are present in nearly equal concentration. At the onset of ripening total soluble sugar content begins to increase rapidly, and the increase is due predominantly to an increase in sucrose; glucose and fructose contents remain fairly constant or may decrease. According to Bianco and Pratt (1977) greater than 97% of the total soluble solids in muskmelon fruit is in the form of soluble sugars, and sucrose accounts for the greatest percentage of the total soluble sugar.

Invertase (β-fructofuranosidase, E.C. 3.2.1.26) catalyzes the hydrolysis of sucrose to glucose and

fructose. Sucrose concentration has been negatively correlated with invertase activity in a variety of fruit types (Beruter 1985, Hawker 1969a, Manning and Maw 1975, Walker and Hawker 1976, Yamaki and Ishikawa 1986). The role of invertase is assumed to be the catalysis of sucrose hydrolysis when the demand for hexose is high (ap Rees 1974, Morris and Arthur 1984). In fruit tissues, invertase activity is often highest during early development, i.e., the period of most rapid growth (Beruter 1985, Walker and Hawker 1976, Yamaki and Ishikawa 1986).

Sucrose synthase (UDPG: D-fructose 2-glucosyl-transferase, E.C. 2.4.1.13) catalyzes the reversible reaction

$$\text{fructose} + \text{UDPG} \rightleftharpoons \text{sucrose} + \text{UDP}.$$

The enzyme is present in a wide variety of plant tissues, but activity is highest in those tissues which are synthesizing starch or cell wall (Pontis 1977, Avigad 1982, Hawker 1985). Sucrose synthase activity in the degradative direction is believed to provide nucleotide sugars which serve as precursors for starch and cell wall components. In cucumber peduncles at least one isozyme of sucrose synthase seems to function in the direction of sucrose synthesis (Gross and Pharr 1982a). In eggplant fruit, sucrose synthase activity has been reported to be an

indicator of mobilizing ability of the tissue (Claussen 1983a, b).

The objectives of this work were to characterize the activity of invertase and sucrose synthase during muskmelon fruit development and determine their relation to soluble sugar composition.

Materials and Method

Plant Material and Sampling

Muskmelons cultivars 'Galia' and 'Noy Yizre'el' were grown at the IFAS Horticultural Unit near Gainesville, Florida. Standard cultural practices for the area were used.

To obtain uniform fruit samples of a known age the method described by McGlasson and Pratt (1963) was followed. Perfect flowers at anthesis were tagged daily. Fruits which set over a three-day period were used as sample material. Samples consisted of ten fruits each and were collected 12, 18, 24, 30, 36, and 42 days after anthesis. Only sound fruit free from defects were included in the samples. Sampling was always done prior to 10 AM. Immediately after the fruit were collected they were taken to the laboratory for processing. The fruit were washed in tap water and then surface sterilized in a solution of sodium hypochlorite (100 ppm). The fruit were then blotted

dry with paper towels. Fresh weights of whole fruit were recorded.

For tissue samples the stem end and blossom end of the fruits were removed and discarded. The remaining equatorial portions of the fruit were sliced into longitudinal sections and subsequently into small pieces (ca. 5 g). The pieces from all ten fruits were combined and thoroughly mixed to provide homogeneous composite samples. The composite samples were stored in sealed polyethylene bags at -20°C.

Extraction of Ethanol-Soluble Sugars

Approximately 15 g of mesocarp tissue was homogenized in 85 ml of 95% ethanol in a Sorvall Omnimixer at 50% maximum speed for 1 min. The homogenates were heated in a boiling water bath for 20 min to inactivate endogenous enzymes. Following cooling, the homogenates were held overnight at -20°C to facilitate precipitation of ethanol-insoluble material. The homogenates were filtered through Miracloth (Calbiochem) and the insoluble material was discarded. The soluble filtrates were stored at -20°C until analyzed for soluble carbohydrates.

Thin Layer Chromatography of Ethanol-Soluble Extracts

Total carbohydrate in ethanol-soluble extracts was estimated using the phenol-sulfuric assay (Dubois et al. 1956) with glucose as a standard. For thin layer chromatography silica gel plates (Redi-Plate Silica gel G)

were preconditioned by spraying with phosphate buffer (20 mM, pH 7.0) and heating at 60°C for 1 hr. Five microliters of ethanol-soluble extract containing approximately 5 mg/ml total carbohydrate were spotted. Five microliters of a standard mixture of glucose, sucrose, raffinose, and stachyose each at 2 mg/ml were also spotted on each plate. Sugars were separated by developing plates in ascending solvent (n butanol-acetic acid ether water 90:60:30:10) for four hours. The carbohydrates were visualized by spraying the plates with 50% H_2SO_4 and heating to 100°C.

Composition Analysis of Ethanol-Soluble Sugars

Individual sugars present in ethanol-soluble extracts were quantified using high pressure liquid chromatography (HPLC) following the method described by Wade and Morris (1982). Aliquots of ethanol-soluble extracts containing approximately 3 mg of total soluble carbohydrate were evaporated to dryness in Reacti-vials (Pierce Chemical Co., Rockford, IL) at 50°C. The extracts were resuspended in 50 µl of the HPLC mobile phase. The mobile phase consisted of acetonitrile-water-tetraethylpentamine (75:25:0.02 v/v).

The HPLC system consisted of a Waters model 510 pump, a Waters U6K injector, a Waters RCM-100 radial compression module, and a Waters 410 refractive index detector. The column used was amine modified silica as described by Hendrix et al. (1981). The flow rate was 2.0 ml/min with a

pressure of 500 p.s.i. Sugars were identified by comparison of retention times with known standards. Sugars were quantified with the external standard method using a Hewlett-Packard 3390-A recorder integrator. Analyses were performed in triplicate and there were three injections of each sample.

Invertase Extraction and Assay

Approximately 25 g of mesocarp tissue was chopped into small pieces and homogenized in 75 ml of ice cold $NaPO_4$ buffer (0.1 M, pH 7.0) for one min in a Sorvall Omnimixer set at 35% maximum speed. The homogenates were squeezed through two layers of cheesecloth and the residue was washed with 10 ml of the extraction buffer. The crude homogenates were centrifuged at 18,000 RPM for 20 min. Supernatants were brought to 80% saturation with dry $(NH_4)_2SO_4$ at 4°C and after standing for 30 min centrifuged at 20,000 RPM for 25 min. Supernatants were decanted and the pellets resuspended in approximately 2 ml of the extraction buffer. Resuspended pellets were centrifuged (clinical at high speed) 15 min; the resulting supernatants were desalted on a column (1.5 cm X 15 cm) packed with Sephadex G-25 which had been equilibrated with $NaPO_4$ buffer (0.1 M, pH 7.0, 0.1 M NaCl). Protein in the fractions was determined by the method of Bradford (1976) with bovine serum albumin used as a standard. Invertase activity was assayed at 35°C in a medium containing 50 µmol Na-acetate

buffer (pH 5.0) 100 µmol sucrose and plant extract in a total volume of 500 µl. The reaction was initiated by adding plant extract to the reaction mixture. The reaction was terminated by the addition of 500 µl of copper-sulfate solution (Somogyi 1952). Test tubes were covered with marbles and heated in a boiling water bath for 20 min. After cooling, 500 µl of arsenomolybdate was added to develop color. Assay mixtures were diluted with 3.0 ml deionized water prior to reading absorbance at 520 nm. Glucose was used as a standard. Data are expressed as µmoles reducing equivalents produced per hour.

Extraction and Assay of Sucrose Synthase

Approximately 10 g of mesocarp tissue was chopped into small pieces and homogenized in 30 ml of ice cold 0.1 M Tris-HCl buffer, 20 mM EDTA, 50 mM cysteine-HCl (pH 8.0) and 2.0% (W/V) PVP, for 1 min in a Sorvall Omnimixer set at 35% maximum speed. The homogenates were squeezed through Miracloth and washed with an additional 5 ml of extraction buffer. The crude homogenates were centrifuged at 18,000 RPM for 20 min. Supernatants were brought to 80% saturation with dry $(NH_4)_2SO_4$ at 4°C and, after standing in an ice water bath for 30 min with occasional stirring, were centrifuged at 20,000 RPM for 25 min. Supernatants were decanted and the pellets resuspended in approximately 2.0 ml of cold extraction buffer. The resuspended pellets were centrifuged in a clinical centrifuge at top speed for

15 min; the resulting supernatants were decanted. Extracts were desalted on a column (1.5 cm x 15 cm) packed with Sephadex G-25 which had been equilibrated with Tris-HCl (50 mM), cysteine-HCl (5 mM) at pH 8.0. One milliliter fractions were collected and protein was determined by the method of Bradford (1976) with bovine serum albumin as the standard. All fractions containing protein were pooled.

Sucrose synthase activity was assayed following the method of Claussen et al. (1984) at 30°C in a medium containing 30 µmoles Tris-HCl (pH 8.0), 5 µmoles UDPG, 15 µmoles fructose, 5 µmoles $MgSO_4$ and 20 µmoles NaF and desalted plant extract in a total volume of 300 µl. The reaction was initiated by adding plant extract to the reaction mix.

The reaction was stopped by the addition of 0.2 ml of 0.2 N NaOH. The test tubes were covered with marbles and heated in a boiling water bath for 10 min to destroy free fructose. Sucrose was determined indirectly by the method of Roe (1934). Sucrose was used as the standard. Boiled controls were included in all experiments. Data are expressed as µmoles sucrose produced per hour.

Results

Fruit Growth

Galia and Noy Yizre'el muskmelon fruits exhibited sigmoidal growth curves (Fig. 1); however, differences

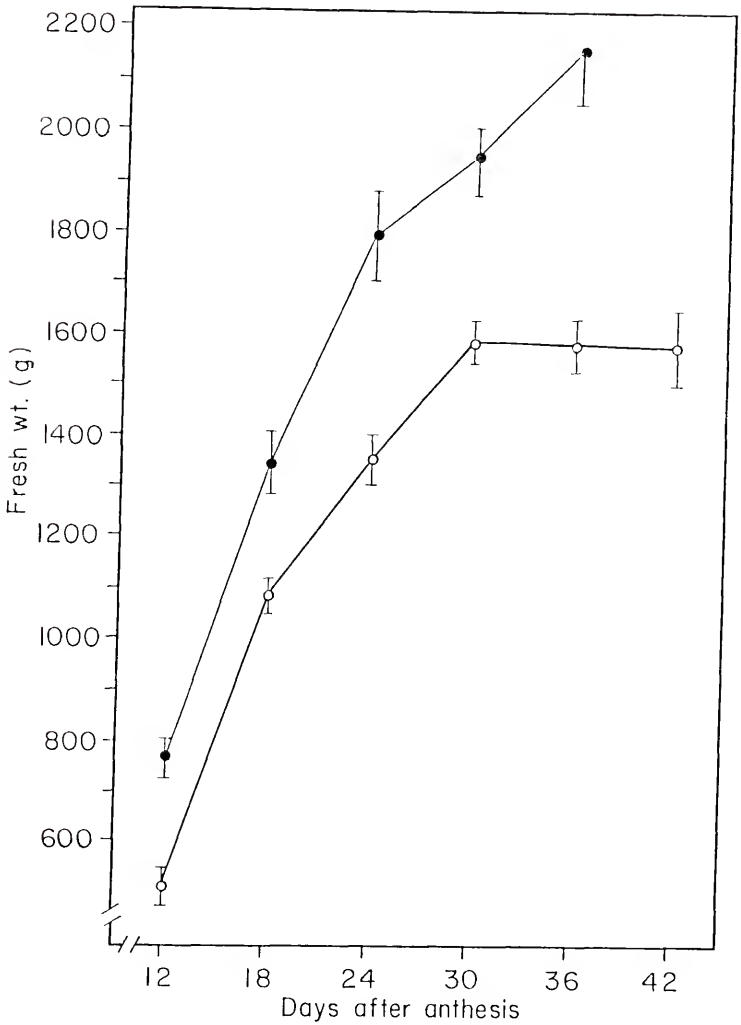

Figure 1. Changes in fresh weight of Galia (●) and Noy Yizre'el (o) muskmelon fruit from 12 days after anthesis until the time of abscission. Values are means of 10 fruit. Vertical bars represent SE of the means.

between the cultivars were apparent. Galia fruits were larger than Noy Yizre'el fruits at each stage of development and continued to accumulate fresh weight until the time of abscission; Noy Yizre'el fruit reached their maximum fresh weight approximately 6 days prior to abscission. Galia fruit abscissed 36 days after anthesis while Noy Yizre'el fruit required 42 days to reach abscission.

Soluble Sugars

Glucose, fructose, and sucrose were the only ethanol-soluble sugars detected in thin-layer chromatographs of mesocarp extracts of both cultivars. Thin-layer chromatography of peduncle and leaf tissue indicated the presence of stachyose and raffinose as well as glucose, fructose, and sucrose in these tissues (data not shown).

The composition of EtOH-soluble sugars during fruit development in the two cultivars was similar (Fig. 2). During the first 24 days after anthesis, glucose and fructose were the predominant EtOH-soluble sugars and were present in nearly equal amounts. Sucrose began to accumulate 24 days after anthesis and accounted for approximately 50% of the total EtOH-soluble sugars at the time of abscission.

Protein Content

The protein content of muskmelon mesocarp tissue ranged from approximately 150-350 $\mu g.g^{-1}$ fresh weight (Fig. 3). In

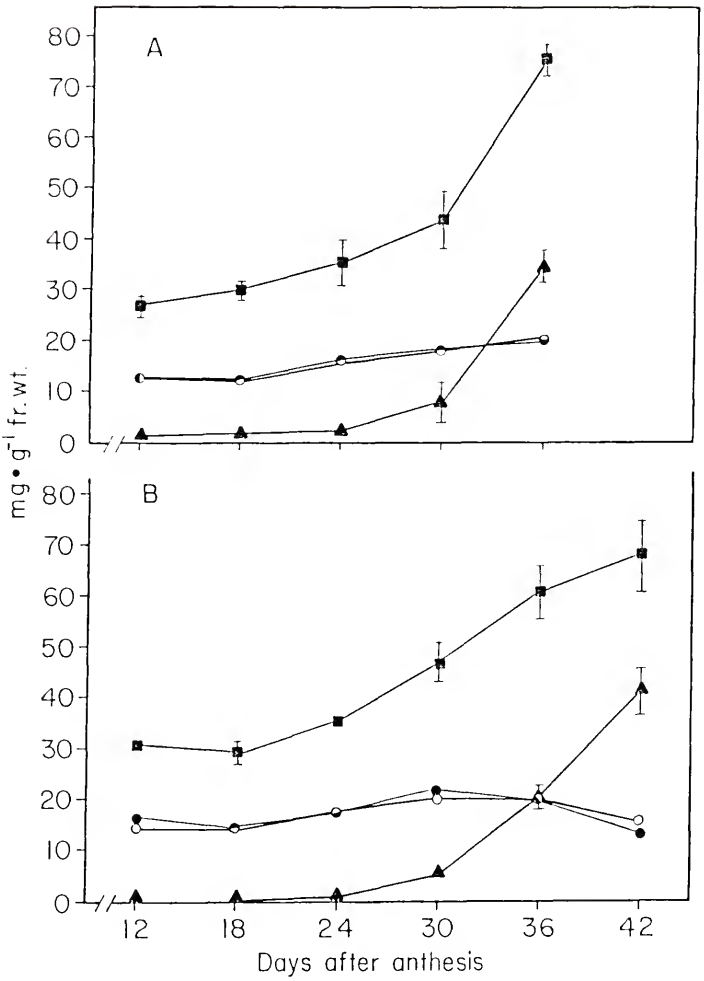

Figure 2. Composition of EtOH-soluble sugars in Galia (A) and Noy Yizre'el (B) mesocarp during development. (■ = total, ● = fructose, ○ = glucose, ▲ = sucrose.) Values are means of three analyses with three injections per sample. Vertical bars represent SE of the means and where absent fall under the symbol.

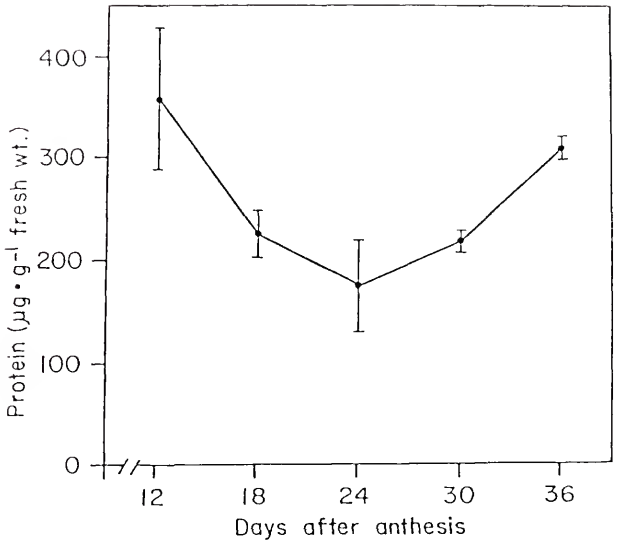

Figure 3. Changes in buffer soluble protein in 'Galia' muskmelon mesocarp during development. Values represent means of three samples. Vertical bars represent SE.

addition, protein content changed with the stage of fruit development. Mean protein content was highest 12 days after anthesis, from 12 to 24 days after anthesis protein content declined, and from 24 days after anthesis until the time of abscission protein content increased.

Invertase Activity

Because acid invertase activity in the two cultivars was similar, only data from Galia are presented. Buffer-soluble extracts from mesocarp homogenates contained an invertase with a pH optimum of 4.0-5.0 (Fig. 4). At pH greater than or less than the optimum, activity decreased rapidly. There was no indication of alkaline invertase activity at any stage of fruit development. The insoluble fraction of the mesocarp homogenates also contained an invertase with a pH optimum of 4.0-5.0 (data not shown); however, activity of the insoluble fraction, on a g fresh weight basis, compared to the activity of the soluble fraction was minimal (< 1%).

Lineweaver-Burk reciprocal plots for acid invertase activity as a function of sucrose concentration were linear (Fig. 5) and indicated a K_m of 4 mM sucrose.

Soluble acid invertase activity, whether expressed on a mg protein or g fresh weight basis, was highest in 12-day old fruit and subsequently decreased with fruit development (Fig. 6). Acid invertase activity in 12-day old fruit was 50-fold greater than in fruit at the time of abscission.

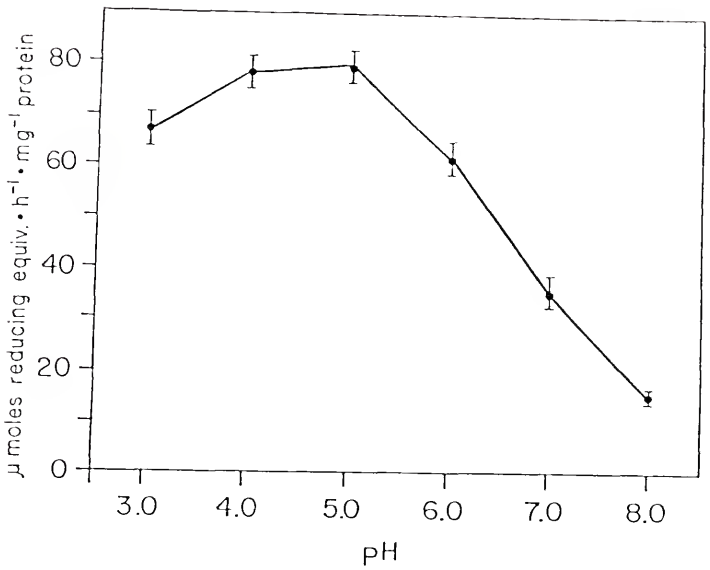

Figure 4. Effect of pH on soluble invertase activity. Values are means of three experiments; each assay was run in triplicate. Vertical bars represent SE of the means.

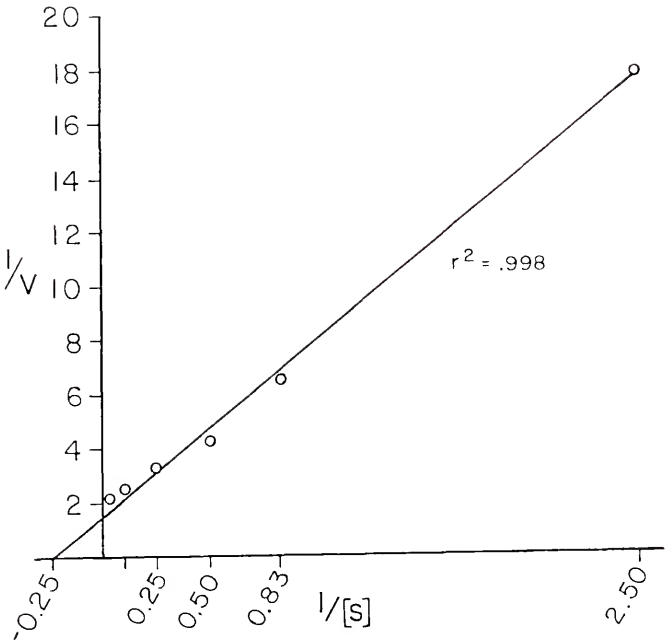

Figure 5. Double reciprocal plots for the effect of sucrose concentration on the activity of soluble invertase at pH 5.0 and 35°C. V: µmoles reducing equivalents; S: sucrose concentration (M).

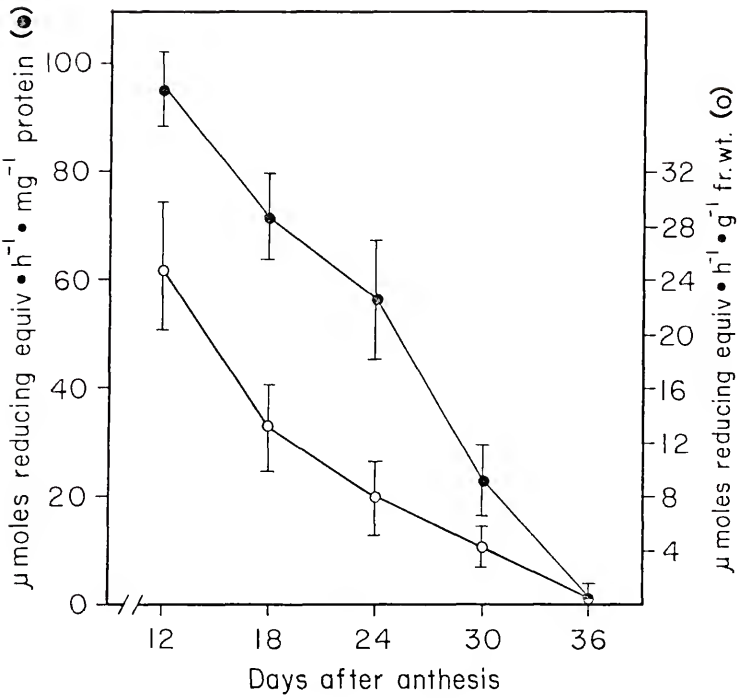

Figure 6. Changes in soluble invertase activity during muskmelon mesocarp development. Values are means of three experiments; each assay was run in triplicate. Vertical bars represent SE of means.

Sucrose Synthase Activity

Sucrose synthase was assayed in the direction of sucrose synthesis using a colorimetric assay (Claussen et al., 1984). Measurement of sucrose synthase activity in the degradative direction indicated that UDPG was formed at a rate roughly equivalent to the rate of sucrose synthesis (data not shown).

The effect of pH on the activity of sucrose synthase in the direction of sucrose synthesis is presented in Fig. 7. Although the rate of sucrose synthesis was maximum at pH 8.0, there were only slight differences over the range of pH 7.0 to 8.0. At pH below 7.0 or above 8.0 sucrose synthase activity decreased rapidly.

Sucrose synthase activity was much lower than invertase activity but increased (ca. 3-fold) during fruit development (Fig. 8). On both a mg protein and g fresh weight basis, the increase in sucrose synthase activity was greatest between 18 and 24 days after anthesis.

Discussion

Fruit growth (Fig. 1) and the composition of EtOH-soluble sugars (Fig. 2) in Galia and Noy Yizre'el muskmelon fruits were similar to those reported for other muskmelon cultivars (Bianco and Pratt 1977, Hughes and Yamaguchi 1983, Lester and Dunlap 1985, Pratt 1971). Reducing sugars

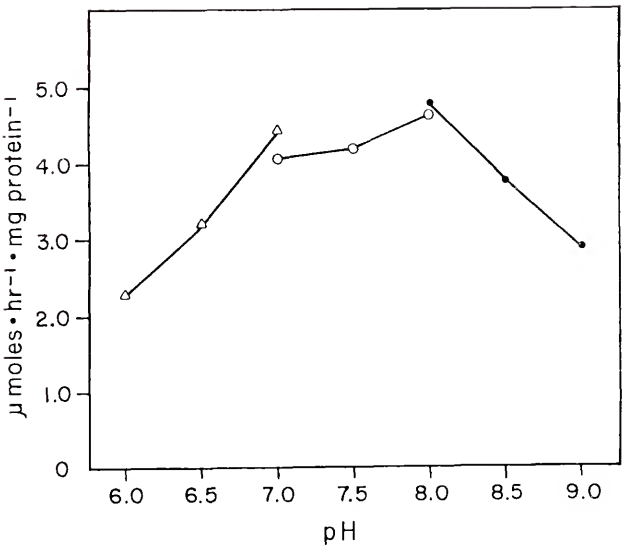

Figure 7. Effect of pH on sucrose synthase (synthetic) activity. (Δ = MES, ○ = HEPES, ● = Tris.) Values represent means of two experiments.

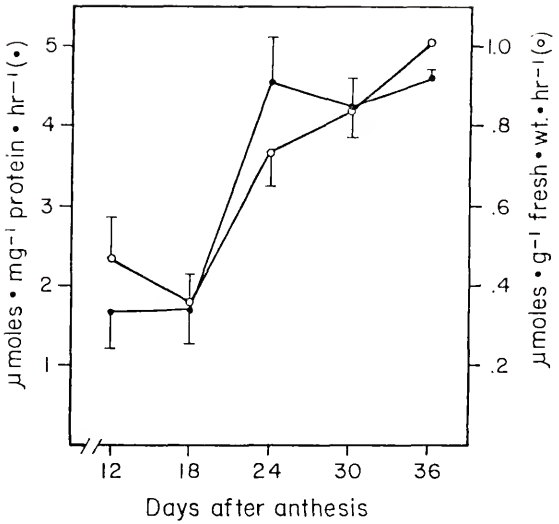

Figure 8. Changes in sucrose synthase (synthetic) activity during muskmelon fruit development. Values are means of three experiments. Vertical bars represent SE of the means.

accounted for greater than 90% of the total soluble sugars during the first 24 days after anthesis, whereas sucrose began to accumulate 24 days after anthesis and accounted for approximately 50% of the total soluble sugars in fruit at the time of abscission.

Buffer-soluble protein content in muskmelon mesocarp averaged 150-350 µg.g freshweight, and changed during fruit development (Fig. 3). During the first 24 days after anthesis there was a continuous decline in protein content. The decrease is most likely due to a dilution effect as a result of fruit growth. From 24 days after anthesis until the time of abscission protein content increased. Rowan et al. (1969) also observed an increase in protein of muskmelon fruit during the ripening process and attributed it to the synthesis of ripening related enzymes.

A soluble acid invertase was present in mesocarp extracts (Fig. 4). A similar invertase has been detected in a number of fruit tissues (Arnold 1965, Kato and Kubota 1978, Manning and Maw 1975). Although alkaline invertase activity has been reported in citrus fruit (Kato and Kubota 1978) as well as other plant tissues (ap Rees 1974), there was no evidence of alkaline invertase activity in muskmelon mesocarp extracts.

Invertase activity in the insoluble fraction of mesocarp homogenates had a pH optimum of 4.0-5.0; however,

on a g fresh weight basis, activity in the insoluble fraction was only a small percent (< 1%) of that in the soluble fraction (data not shown). It may, therefore, be concluded that acid invertase in muskmelon mesocarp cell is not bound or only loosely bound to the cell wall. This finding is in agreement with that reported for grape berry (Hawker 1969) and citrus fruit invertase (Kato and Kubota 1978). In contrast, Walker and Hawker (1976) found high insoluble acid invertase activity in young watermelon and pepper fruits. The conditions of extraction are known to have an effect on the binding of invertase to the insoluble fraction (Hawker 1969), and this may be the reason for the difference in insoluble invertase activity. The high solubility and acid pH optimum indicate a vacuolar location for muskmelon mesocarp invertase.

The K_m value of 4 mM as determined by Lineweaver-Burk reciprocal plots (Fig. 5) is similar to that reported for acid invertase from grape berries (Arnold 1965), citrus fruit (Kato and Kubota 1978) and other plant tissues.

Invertase activity during muskmelon fruit development declined continuously from 12 days after anthesis until the time of abscission (Fig. 6). This trend is in contrast with the activity of acid invertase in grape berries in which activity increased to a maximum about midway through development and thereafter remained fairly constant (Hawker 1969). In tomato, acid invertase activity increased during

fruit development and reached a maximum in overripe fruit (Manning and Maw 1975). However, in contrast with muskmelon, grape berries (Hawker 1969) and tomatoes (Manning and Maw 1975) have very low sucrose contents.

In muskmelon mesocarp the activity of acid invertase was negatively correlated with sucrose content (Figs. 2 and 6). This is similar to that reported for apple fruit (Beurter 1985, Yamaki and Ishikawa 1986), grape berries (Hawker 1969), tomato fruit (Manning and Maw 1975), and citrus fruit (Kato and Kubota 1978). In some tissues reducing sugar content has shown a positive correlation with invertase activity (Walker and Hawker 1976); however, in muskmelon mesocarp reducing sugar content was not correlated with invertase activity (Figs. 2 and 6).

The activity of acid invertase was highest during early fruit growth when the sucrose content was lowest. These results indicate that sucrose may be the source of hexoses required for metabolism and that high invertase activity prevents sucrose from accumulating. Additionally, invertase may be acting to hydrolyze sucrose to glucose and fructose in order to maintain osmotic pressure of the cell. High osmotic pressure is required for the accumulation of water during fruit growth. During the latter stages of fruit development, as growth slows invertase activity is apparently low enough to allow sucrose to accumulate.

It is known that invertase is required for the import of sugars in a variety of plant tissues. This does not appear to be the case in muskmelon mesocarp because invertase activity is low during the period when soluble sugar accumulation is rapid.

Sucrose synthase activity was also detected in buffer-soluble extracts prepared from muskmelon mesocarp. Because sucrose synthase catalyzes a reversible reaction it is not possible, based on these experiments, to determine which direction the reaction was proceeding *in vivo*. In most cases it is assumed that sucrose synthase is responsible for sucrose degradation (ap Rees 1974, Avigad 1982, Hawker 1985). However, in cucurbit peduncles it has been reported that sucrose synthase may act in the direction of sucrose synthesis (Gross and Pharr 1982).

Sucrose phosphate synthase (UDPG: D-fructose 6-P 2-glucosyltransferase, E.C. 2.4.1.14) activity was not assayed in these studies, nor was the ability of the mesocarp to synthesize sucrose from exogenously applied hexoses. Therefore it is not known whether the tissue is synthesizing sucrose or merely accumulating sucrose during the latter stages of development. The pattern of invertase (Fig. 6) and sucrose synthase (Fig. 8) activities in muskmelon mesocarp are very similar to those reported for sugar beet storage roots (Giaquinta 1979). Sucrose accumulation in muskmelon mesocarp may be regulated in a

way similar to that proposed for sugar beet storage roots. If that is the case, sucrose would accumulate without hydrolysis and resynthesis in the mesocarp.

CHAPTER IV
FRUIT SOFTENING

Introduction

Loss of flesh firmness or softening is a general characteristic of the ripening process for a variety of fruit types. Softening results from a dissolution of cell wall components, particularly the pectic polysaccharides (Pilnik and Voragen 1970, Pressey 1977, Huber 1983a). Rosa (1928) investigated compositional changes in muskmelon fruit during ripening and found a conversion of insoluble protopectin to soluble pectin and pectic acid. Dinus and Mackey (1974) concluded that flesh texture in muskmelons was related to insoluble pectin and starch content. Solubilization of pectins is frequently attributed to the action of D-galacturonases (Huber 1983a, Pressey 1977). However, two reports (Hobson 1962, Lester and Dunlap 1985) have indicated that muskmelon fruit lack D-galacturonase activity. The absence of D-galacturonase indicates that some other mechanism must be responsible for pectin solubilization in muskmelon fruit.

In addition to the degradation of pectins by D-galacturonases, other modifications of cell wall polymers may be involved in fruit softening. A net loss of cell wall neutral sugar occurs during softening in a variety of

fruit types (Knee 1973b, Knee et al. 1977, Wallner and Bloom 1977, Yamaki et al. 1979, Ahmed and Labavitch 1980, Gross and Sams 1984). Arabinose and galactose were the major neutral sugars lost during fruit softening in 14 of 17 species examined by Gross and Sams (1984). Ahmed and Labavitch (1980) suggested that the loss of arabinose during softening in pear was the result of D-galacturonase activity. In tomato fruit, D-galacturonase does not appear to be the enzyme responsible for the removal of arabinose and galactose (Gross and Wallner 1977, Wallner and Bloom 1979). Arabinose and galactose are components of hemicelluloses as well as pectin, and modification of hemicelluloses may be related to softening. Yamaki et al. (1979) found a loss of arabinose and galactose from hemicellulose during pear fruit softening. Huber (1983b, 1984) has shown that hemicelluloses from tomato and strawberry fruit are modified during ripening in a pattern that indicates degradation. A similar modification of hemicelluloses has been reported to occur during ripening of hot pepper fruit (Gross et al., 1986).

The objectives of the studies presented here were to investigate the changes in pectins and hemicelluloses during muskmelon fruit softening in order to gain a better understanding of how metabolism of these wall components might contribute to softening. Specifically, pectin solubility and molecular size and hemicellulose molecular size and composition were determined. Additionally,

D-galacturonase activity and hemicellulase activity were investigated. Noncellulosic neutral sugar composition of ethanol-insoluble solids was also measured.

Materials and Methods

Plant Material

Muskmelons (Cucumis melo var. reticulatus Naud. cv. 'Galia') were grown at the IFAS Horticultural Unit near Gainesville, FL. Fruit were selected based on visual indication of ripeness and separated into 3 categories: 1) preripe-full size but prior to the initiation of ripening, 2) ripe-fruits which were at the full slip (abscission) stage, 3) overripe-fruits which were harvested at the ripe stage and stored for 6 days at 22°C. Only sound fruits free from defects were used. The placental contents were discarded and the pericarp cut into uniform pieces. The tissue was stored frozen (-20°C) in sealed polyethylene bags until used.

Flesh Firmness

Flesh firmness was measured as resistance to puncture using a Magness Taylor pressure tester with a 1.1 cm tip on two pared areas opposite each other on the equatorial region of the fruit. The data are expressed in Newtons (N).

Preparation of Ethanol-Insoluble Powders (EIP) and Cell Wall (CW)

Approximately 200 g of mesocarp tissue were homogenized in 95% EtOH (800 ml) for 4 min in a blender on

high speed. Homogenates were heated in a boiling water bath for 30 min. to inactivate endogenous enzymes. Following boiling, the homogenates were held overnight at -20°C to facilitate precipitation of EtOH-insoluble compounds. The insoluble material on glass fiber filters (Whatman GF/C) was washed with acetone (21) and then dried under vacuum at room temperature.

Cell wall was prepared by homogenizing approximately 100 g of mesocarp tissue in 250 ml ice cold water in a blender on high speed for 4 min. Homogenates were filtered through Miracloth (Calbiochem) and washed with ice cold water (31) followed by cold acetone (1 l). Acetone washed homogenates were filtered through glass fiber filters (Whatman GF/C) and the insoluble material (cell wall) dried under vacuum at room temperature.

Polyuronide Extraction, Quantification, and Gel Chromatography

Total polyuronides in EIP and CW were determined as described by Ahmed and Labavitch (1977). Approximately 7 mg EIP or CW were chilled in beakers placed in an ice bath. Cold concentrated H_2SO_4 (2.5 ml) was added and the samples stirred for 4 min. Cold water (0.7 ml) was added dropwise followed by a 5 min incubation. A second aliquot of cold water (0.7 ml) was added followed by a second 5.0 min incubation. Following hydrolysis the volume of the extracts was adjusted to 25 ml with cold water. Total

polyuronides were determined (Abs 520) using the procedure of Blumenkrantz and Asboe-Hansen (1973). Values are expressed as µg galacturonic acid equivalents per mg powder.

Soluble polyuronides were determined by incubating approximately 20 mg EIP or CW in 7 ml Na-acetate-acetic acid buffer (30 mM, pH 5.0) without Na_2-EDTA (buffer soluble) or with Na_2-EDTA (chelator soluble). The suspensions were incubated at 23°C for 24 hrs. At time zero and intervals throughout the incubation 0.5 ml aliquots were removed, filtered, and assayed for acid sugars (Blumenkrantz and Asboe-Hansen 1973).

For gel chromatography of polyuronides, approximately 20 mg of EIP or CW was suspended in 2 ml of Na-acetate-acetic acid buffer (40 mM, pH 5.0) plus 20 mM Na_2-EDTA. The suspensions were incubated at 23°C for 6 h with continuous stirring. Following incubation, the suspensions were filtered through Miracloth and washed with 1 ml of buffer, centrifuged (clinical, high speed, 10 min), and filtered through glass fiber filters (Whatman GF/C). Gel-chromatography was carried out on a bed (60 cm high, 1.5 cm dia.) of Ultrogel AcA 22 (Bio-Rad Lab., Richmond, CA) packed in Na-acetate-acetic acid buffer (40 mM pH 5.0) plus 100 mM NaCl and 20 mM Na_2-EDTA. The sample (approximately 2.5 ml) was gravity eluted with a head pressure of 60 cm. Fractions of 2 ml were collected and 0.5 ml of each

fraction was assayed for acid sugars using the method of Blumenkrantz and Asboe-Hansen (1973).

Extraction and Assay of Polygalacturonase

Polygalacturonase (E.C. 3.2.1.15) (PG) was extracted by homogenizing approximately 40 g of partially thawed mesocarp tissue in 40 ml of ice cold Na-acetate buffer (0.1 M pH 4.5) plus 2 M NaCl. The tissue was homogenized for 1 min in a Sorvall Omnimixer set at half maximum speed. Homogenates were held on ice for 1 h, stirred occasionally, then filtered through two layers of cheesecloth. The filtrates were centrifuged 20 min at 18,000 RPM at 4°C. Supernatants were brought to 80% saturation with dry ammonium sulfate and held in an ice water bath for 1 h. Saturated extracts were then centrifuged at 18,000 RPM for 25 min at 4°C. Supernatants were decanted and the pellets were resuspended in 2 ml of the extraction buffer and centrifuged (clinical, high speed) for 15 min. The supernatants were decanted and subsequently desalted on a column (1.6 cm x 15 cm) packed with Sephadex G-25 which had been equilibrated with Na-acetate buffer (50 mM pH 4.5 plus 150 mM NaCl). Protein was determined by the method of Bradford (1976) with bovine serum albumin as the standard.

For the assay of PG activity, ethanol-purified polygalacturonic acid (2 mg galacturonic acid equivalents/ml in 30 mM Na-acetate buffer pH 4.5 plus

150 mM NaCl and 0.01% thimerosal) was used as the substrate.

The reaction was initiated by addition of 50 µl of desalted protein preparation to 500 µl of substrate. The reaction mixture was incubated at 32°C for up to 19 h in a shaking water bath. The reaction was stopped by the addition of 1.5 ml of the Milner-Avigad (1967) copper sulfate solution. The tubes were covered with marbles and heated in a boiling water bath for 10 min. After cooling in an ice water bath, color was developed by the addition of 500 µl of arsenomolybdate reagent (Somogyi 1952), and absorbance at 600 nm was measured. Galacturonic acid was used as the standard. Data are expressed as µmoles galacturonic acid equivalents released per mg protein per minute.

Analysis of Cell Wall Autolytic Activity

For autolysis experiments approximately 70 mg cell wall was suspended in 20 ml 80% EtOH. Cell wall suspensions were either heated in a boiling water bath for 20 min to inactivate endogenous enzymes or held at room temperature. Ethanol was removed by filtering through Miracloth followed by washing with 275 ml of deionized water. The cell wall material was then resuspended in 5 ml Na-acetate buffer (40 mM plus 100 mM NaCl, pH 4.5) which contained 0.02% (w/v) thimersol to prevent microbial growth. The wall material was incubated in a shaking water

bath at 35°C for 8 h. At the end of the incubation the wall material was filtered through Miracloth and washed with an additional 15 ml of the buffer. The soluble extracts were then dialyzed overnight against running deionized water. Following dialysis the extracts were filtered through glass fiber filters (Whatman GF/C) and the volume was reduced to approximately 2.0 ml. These extracts were then subjected to Ultrogel AcA 22 chromatography as described previously.

Starch Determination in Ethanol-Insoluble Powders

Starch determination was performed in two separate experiments. In the first experiment approximately 15 mg ethanol-insoluble powder were suspended in 2 ml acetate buffer (0.1 M, pH 4.5). The suspensions were heated in an 85°C water bath for 15 min to gelatinize starch. After cooling to 37°C, 50 µl of a suspension of amyloglucosidase (Rhizopus, Sigma) was added and the suspensions incubated at 37°C for 8 h. Following incubation, the suspensions were filtered through glass fiber filters (Whatman GF/C) and glucose determined by the glucose oxidase method. The difference in glucose between boiled and active amyloglucosidase was taken as starch.

The second method of starch determination was based on the method described by Loescher and Nevins (1972). Approximately 30 mg of ethanol-insoluble powders were suspended in 2 ml of HEPES buffer (15 mM, pH 6.9 plus 5 mM

NaCl). The suspensions were heated in an 85°C water bath for 15 min to gelatinize starch. After cooling to 37°C, 50 µl of a suspension of α-amylase (porcine pancreatic, Sigma Type 1-A) in HEPES (15 mM, pH 6.9) were added and the suspensions were incubated at 37°C for 8 h. The soluble filtrates were then evaporated to dryness in Pierce Reacti-vials. Soluble carbohydrates were hydrolyzed using 2 N trifluroacetic acid as described by Jones and Albersheim (1972) and the monomers were converted to alditol acetates. Alditol acetates were separated using gas chromatography.

Hemicellulose Extraction, Gel Chromatography, and Compositional Analysis

Approximately 500 mg of EIP were suspended in $NaPO_4$ buffer (20 mM, pH 6.8) and held in a boiling water bath for 30 min. Following boiling the EIP suspensions were filtered through Miracloth, and the material retained was saved. The insoluble material was resuspended in 10 ml 4 N KOH plus $NaBH_4$ (5 mg/ml), and stirred overnight. The solutions were filtered through Miracloth and the residue rinsed with 2 ml deionized water. The pH of the filtrates was adjusted to 7.0 with acetic acid. The extracts were dialyzed against running tap water overnight, MeOH (10%, 24 hr, 4 l, 2X) and deionized water (overnight, 4 l, 3X). Total carbohydrate in the extracts was determined by the method of Dubois et al. (1956).

For gel chromatography approximately 3-5 mg of hemicellulose in 2 ml was applied to a bed (60 cm high, 1.5 cm wide) of Ultrogel AcA 34 (Bio-Rad Lab., Richmond, CA) packed in Na-acetate-acetic acid buffer (40 mM, pH 5.0) containing 100 mM NaCl and 5 mM Na_2-EDTA. Fractions of 2 ml were collected and analyzed using the phenol-sulfuric procedure (Dubois et al. 1956).

For compositional analysis of hemicellulose a volume equivalent to 2 mg of total sugar was air dried in Reacti-vials (Pierce Chemical Co., Rockford, IL). For gas chromatography the polymers were hydrolyzed using 2N TFA and acetylated following the procedure of Loescher and Nevins (1972). Chromatography of acetylated sugars was conducted on a column packed with SP 2340 (Supelco Co. Inc., Bellefonte, PA) with myoinositol as an internal standard. Analyses were performed twice. Each analysis consisted of triplicate injections of each sample.

Results

Flesh Firmness

Muskmelon flesh firmness, measured as resistance to puncture, decreased markedly during ripening (Fig. 9). There was a 60% decrease in firmness from the preripe to ripe stage and a further decrease of 56% from ripe to overripe.

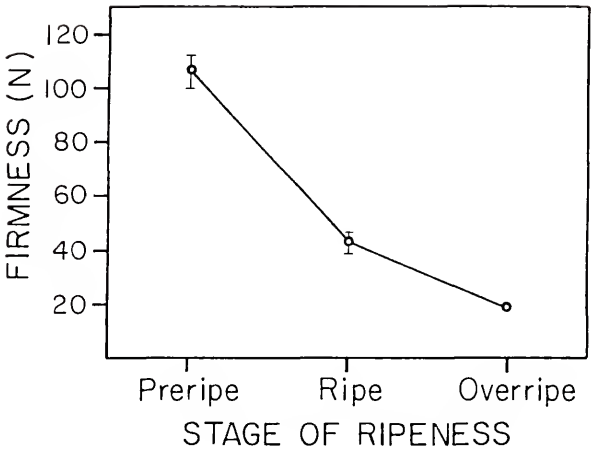

Figure 9. Firmness of 'Galia' muskmelon fruit at three stages of ripeness. Values represent means of 20 measurements. Vertical bars represent SE and, where absent, fall under the symbol.

Yield of EIS and Cell Wall

Ethanol-insoluble solids and cell wall averaged 10.6 $mg.g^{-1}$ fresh weight and 6.3 $mg.g^{-1}$ fresh weight, respectively, of muskmelon mesocarp (Table 1). The mean yield of EIS increased 27% from the preripe to ripe stage and then remained constant from the ripe to overripe stage. In contrast to EIS, mean cell wall yield showed a consistent (13%) decrease from the preripe to overripe stage.

Polyuronides

Although total pectins in ethanol-insoluble solids remained fairly constant during ripening, there was a slight (7%) decrease from preripe to overripe (Table 2). Total pectins in the cell wall showed a less consistent pattern of change, but also decreased slightly from the preripe to overripe stage. There were significant increases in the quantities of water-soluble and Na_2-EDTA-soluble polyuronides in ethanol-insoluble powders during the transition from the preripe to ripe (Figs. 10A and B), whereas little further increase occurred during the transition from ripe to overripe. Water-soluble polyuronides represented approximately 19%, 40%, and 41% of the total ethanol-insoluble pectins from preripe, ripe, and overripe tissues, respectively. Chelator had a pronounced effect on pectin solubility. Chelator-soluble polyuronides accounted for approximately 40%, 68%, and 72% of the total

Table 1. Yield of ethanol-insoluble solids (EIS) and cell wall.

Stage	EIS	Cell wall
	$mg.g^{-1}$ fr.wt.	
Preripe	9.25±0.49[z]	6.70±0.30
Ripe	11.73±0.79	6.47±0.45
Overripe	10.99±0.38	5.85±0.28

[z] Values represent means ± SE for 3 separate preparations of each sample.

Table 2. Total pectins in ethanol-insoluble solids (EIS) and cell wall.

Stage	EIS	Cell wall
	$\mu g.g^{-1}$ dry wt.	
Preripe	234.7±10.9[z]	321.8±23.2
Ripe	224.0±13.1	345.8± 7.0
Overripe	217.0± 6.5	304 ±14.5

[z] Values represent means ± SE for 3 replicates of each sample.

Table 3. Hemicellulose content of ethanol-insoluble solids.

Stage	$mg.100\ mg^{-1}$ EIS
Preripe	4.4±0.50[z]
Ripe	4.0±0.25
Overripe	4.0±0.12

[z] Values represent means ± SE of 3 replicates.

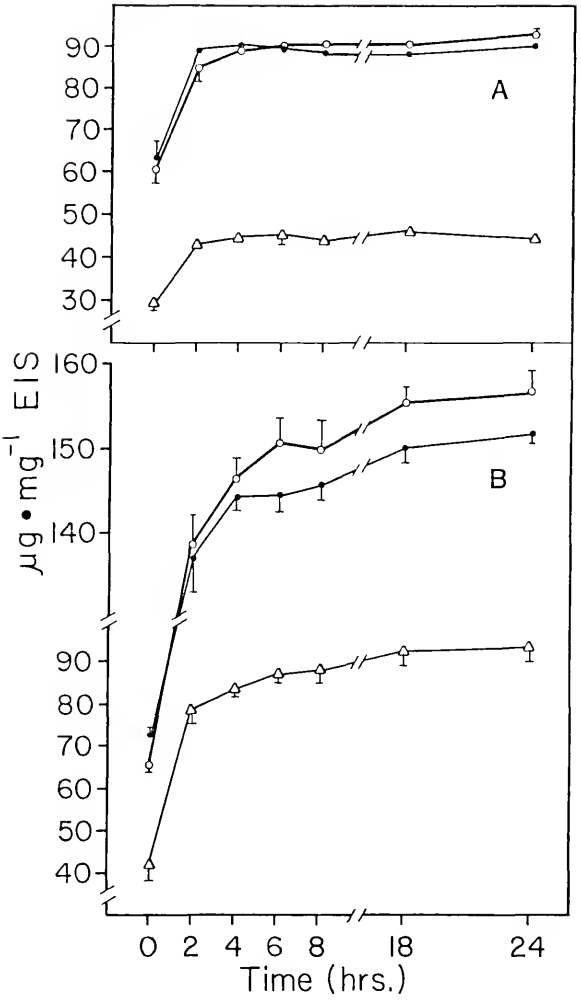

Figure 10. Extraction of water-soluble (A) and Na_2-EDTA soluble (B) polyuronides from ethanol insoluble powder. (Δ = preripe, ● = ripe, o = overripe.) Values are means of three replicates. Vertical bars indicate SE and when absent fall under the symbol.

ethanol-insoluble pectins from preripe, ripe, and overripe tissues, respectively.

The quantity of Na_2-EDTA-soluble pectins in cell wall also increased with ripening (Fig. 11). Chelator-soluble pectins represented 28% of the total pectins in cell wall from preripe tissue. In ripe and overripe tissue chelator-soluble pectins increased to 55% of the total pectins.

The molecular size of chelator-soluble polyuronides from ethanol-insoluble solids tended to decrease with ripening (Fig. 12). A similar trend was detected in the molecular size of chelator-soluble pectins from cell walls (Fig. 13).

The change in pectin size was apparently not due to the action of D-galacturonase. No D-galacturonase activity was detected in NaCl-soluble extracts prepared from melon mesocarp. Although D-galacturonase activity was not detected in muskmelon mesocarp extracts, it was possible that the enzyme was inhibited or inactivated during the extraction procedure. To test these possibilities muskmelon tissue was cohomogenized with tomato pericarp, a rich source of D-galacturonase. D-Galacturonase activity in the cohomogenates was similar to activity in tomato extracts alone. Additionally, muskmelon cell wall incubated in buffer did not release pectin fragments enzymically (autolysis). Lack of autolytic activity indicates that PG was not present in the insoluble fraction.

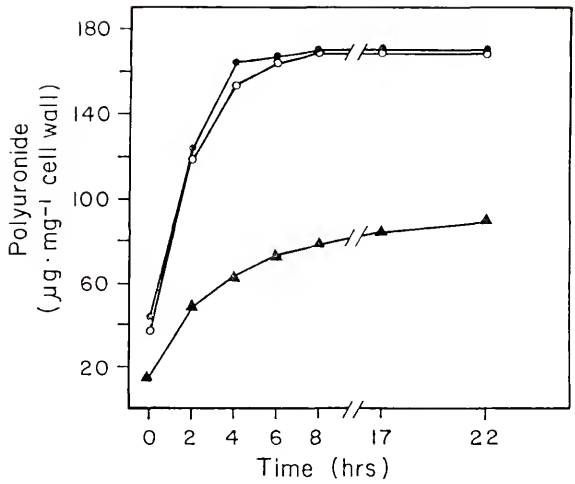

Figure 11. Extraction of Na_2-EDTA-soluble polyuronides from isolated cell wall. (Δ = preripe, ● = ripe, o = overripe.)

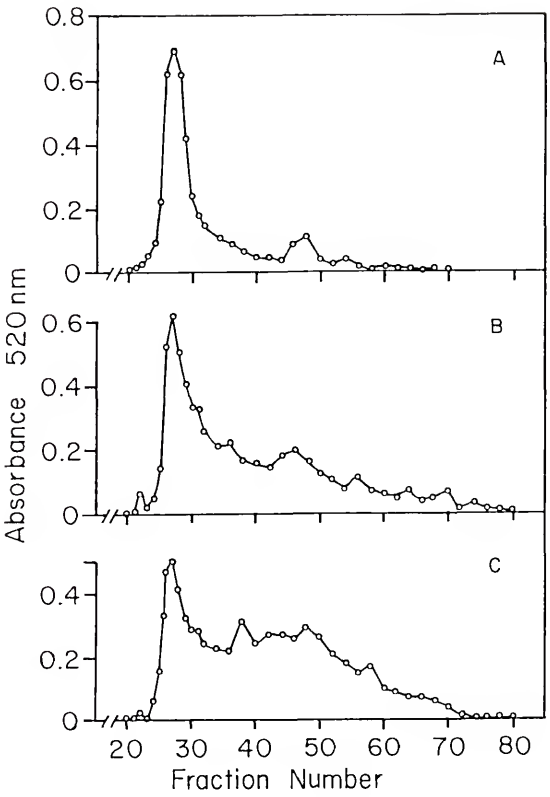

Figure 12. Ultrogel AcA 22 profiles of polyuronides extracted from ethanol-insoluble powders. (A = preripe, B = ripe, C = overripe.)

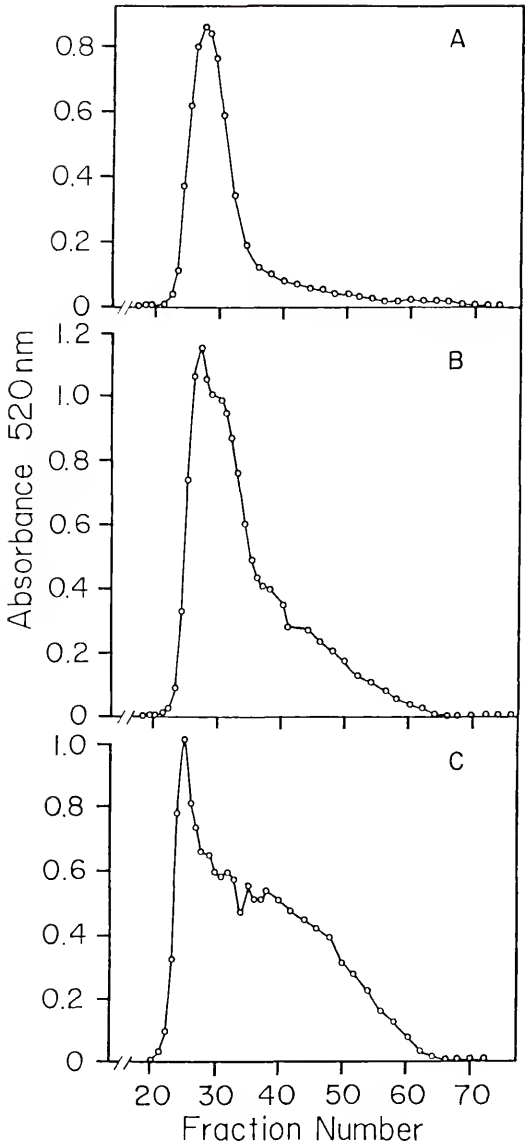

Figure 13. Ultrogel AcA 22 profiles of polyuronides extracted from isolated cell wall. (A = preripe, B = ripe, C = overripe.)

Hemicelluloses

Hemicelluloses (defined as that fraction soluble in 4 N NaOH) represented approximately 4% of the ethanol-insoluble powders in the preripe to ripe stage (Table 3). The molecular weight distribution of hemicelluloses shifted during ripening (Fig. 14). Hemicelluloses from preripe tissue were predominantly large molecular weight polymers (voided Utrogel AcA 34). As ripening progressed there was a decrease in large polymers and a relative increase in polymers retained by the gel. Based on results obtained with amylase treated hemicelluloses, the changes in hemicellulose were not due to starch. No difference in the elution profiles of amylase-treated and nonamylase-treated hemicelluloses was detected. Acid sugars constituted only a small percentage (<10%) of the total sugars in hemicellulose extracts (data not shown).

Hemicellulosic neutral sugar composition changed during ripening (Table 4). Glucose, galactose, and xylose were the predominant neutral sugars in hemicellulose extracts at all stages of development, while smaller amounts of arabinose and mannose were also present. On a mole-percent basis, glucose and galactose decreased 8% and 32%, respectively, and xylose increased 22% from preripe to overripe. In an effort to characterize further the changes in neutral sugar composition, hemicelluloses, following gel chromatography, were separately combined into two

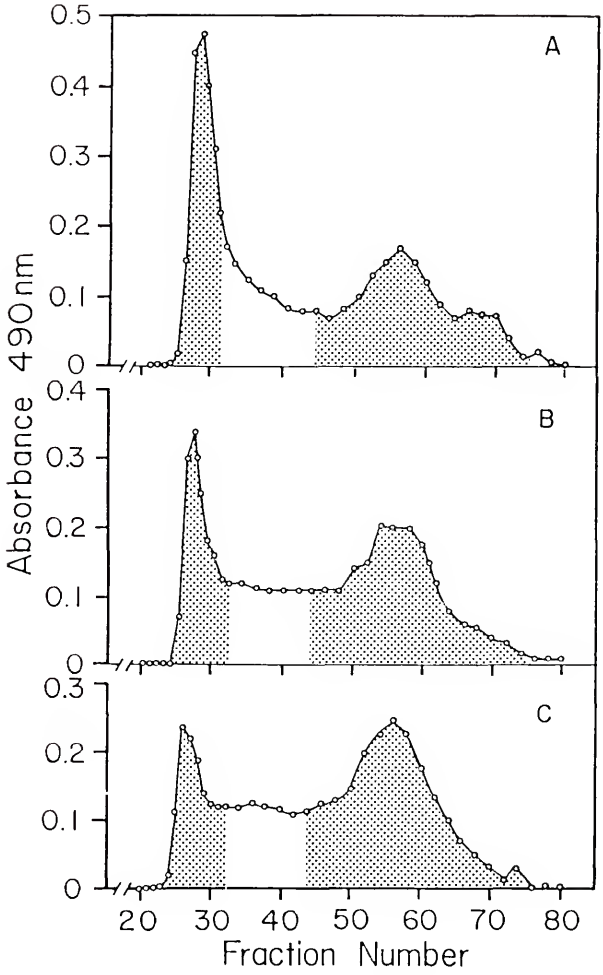

Figure 14. Ultrogel AcA 34 profiles of hemicelluloses extracted from ethanol-insoluble powders. (A = preripe, B = ripe, C = overripe.) Stippled areas indicate void material and inclusion material which were pooled for compositional analyses.

Table 4. Compositional analyses of 'Galia' muskmelon fruit hemicelluloses. Hemicelluloses were analyzed prior to and following Ultrogel AcA 34 chromatography. Values are expressed on a mole-percent basis.

Stage of ripeness	Hemicellulose Fraction[a]	Neutral Sugar (mole-%)				
		Ara	Xyl	Man	Gal	Glu
Preripe	Nonfractionated	6.5	32.7	0.9	19.6	40.2
	Void	6.1	26.1	1.5	34.6	31.5
	Retained	4.8	50.0	1.6	9.5	32.5
Ripe	Nonfractionated	6.6	37.7	0.9	17.9	36.8
	Void	9.0	34.0	ND[b]	33.0	22.0
	Retained	5.2	47.8	0.9	12.2	30.4
Overripe	Nonfractionated	7.7	39.8	1.9	13.2	36.9
	Void	9.8	52.1	ND	16.9	19.7
	Retained	5.4	47.7	1.0	11.7	30.6

[a] Nonfractionated samples were not separated; void and retained samples represent fractions 20-32 and 44-75, respectively, which were pooled following Ultrogel AcA 34 chromatography.

[b] ND = none detected.

fractions: large molecular size (void material) and small molecular size (inclusion material). In the large polymers, glucose and galactose content decreased 37% and 51%, respectively, and xylose increased 50%. In the polymers retained by the gel, xylose was the predominant neutral sugar and accounted for nearly 50% of the total. Glucose decreased 6% and galactose increased 23% in the small polymers as ripening progressed.

Neutral Sugar Composition of Ethanol-Insoluble Solids

There was a net decrease in the noncellulosic neutral sugar content of ethanol-insoluble solids during ripening (Table 5). As with isolated hemicelluloses, glucose, galactose, and xylose were the predominant neutral sugars with smaller amounts of rhammnose, arabinose and mannose also detected. Glucose content increased from the preripe to ripe stage, and then decreased from the ripe to overripe stage. Galactose decreased consistently from the preripe to overripe stage, as did mannose and rhammnose. Arabinose and xylose had no consistent change during ripening.

Treatment of hemicelluloses prepared from preripe tissue with NaCl soluble protein extracted from ripe tissues resulted in a change in hemicellulose molecular weight similar to that observed *in situ* (Fig. 15). This change indicated that hemicellulose degradation is enzymically mediated.

Table 5. Noncellulosic neutral sugar composition of 'Galia' muskmelon ethanol-insoluble powders during ripening.

Stage	Rha	Ara	Xyl	Man	Gal	Glu	Total
Preripe	0.83±0.7[z]	1.74±0.39	3.26±0.1	1.77±0.7	7.61±1.4	4.94±1.9	22.3±8.4
Ripe	0.60±0.7	2.15±0.52	3.90±1.38	0.90±0.4	2.68±0.9	7.58±2.1	16.7±6.2
Overripe	0.47±0.2	1.86±0.74	2.78±1.4	0.67±0.1	1.54±0.1	3.28±0.6	9.34±0.8

[z] Data represent means ± SD of 3 separate analyses.

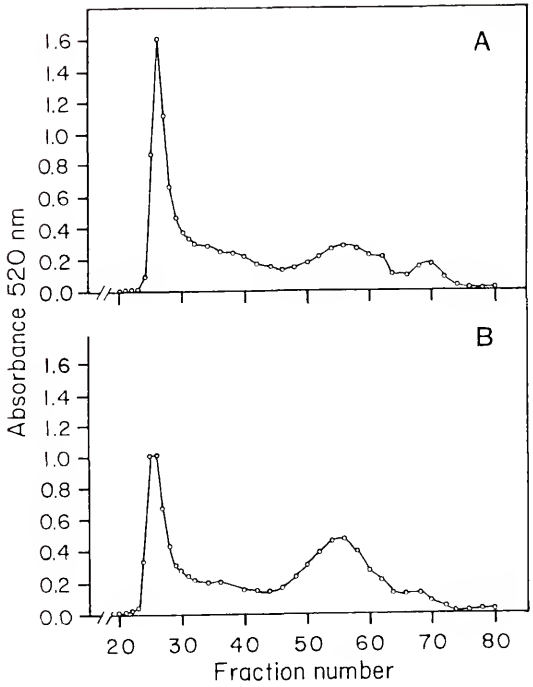

Figure 15. Ultrogel AcA 34 profiles of preripe hemicelluloses following incubation in buffer (A) or salt soluble protein from ripe tissue (B).

Discussion

Muskmelon fruit softened extensively during ripening (Fig. 9). Softening commenced approximately 24 days after anthesis and was most pronounced during the transition from preripe to ripe, although additional softening occurred as the fruits became overripe. A similar pattern of softening has been reported for 'Perlita' muskmelon fruit (Lester and Dunlap 1985).

Fruit softening is generally attributed to a dissolution of cell wall components (Pressey 1977, Huber 1983). During softening of tomato (Wallner and Bloom 1977, Rushing 1985) and pear (Ahmed and Labavitch 1980) this dissolution of cell wall components is reflected in an increase in the quantity of water-soluble cell wall material. A similar loss in cell wall material took place during muskmelon fruit softening (Table 1). In contrast with cell wall, the quantity of ethanol-insoluble solids increased during the transition from preripe to ripe, and then remained constant. In strawberry (Huber 1984), there was also an increase in the yield of ethanol-insoluble solids on a per fruit basis, but not on a gram fresh weight basis. The increase in yield of ethanol-insoluble powders may reflect an increase in some component other than cell wall polysaccharides. For example, protein increases during muskmelon fruit ripening (Chapter III) and would contribute to the increase in ethanol-insoluble material.

Total pectins in ethanol-insoluble powders and cell wall decreased only slightly during ripening (Table 2). Lester and Dunlap (1985) reported no change in total pectins as a percentage of cell wall material in 'Perlita' muskmelon fruit. In tomato (Huber and Lee 1986) and strawberry (Woodward 1972, Huber 1984) it has been shown that total pectins in ethanol powders increase during ripening. Quantitative changes in pectin content are apparently less important in the softening process than are qualitative changes.

The quantity of soluble pectins in ethanol powders (Fig. 10) and cell wall (Fig. 11) increased as muskmelon fruit softened. As with the loss of firmness, the greatest change in pectin solubility occurred during the transition from preripe to ripe. Rosa (1928) also reported that the solubility of pectins increases during muskmelon fruit ripening. Pectin solubility was enhanced in the presence of EDTA (Fig. 10), a strong calcium chelator. Pectin solubility has been enhanced with calcium chelators in other fruit tissues (Jarvis 1982). Neal (1965) suggested that increased pectin solubility in strawberry fruit was the result of a decrease in calcium in the cell wall. Lester and Dunlap (1985) investigated changes in calcium during muskmelon fruit ripening and found no change in the quantity of soluble or bound calcium. Although removal of

calcium ions enhanced pectin solubility in muskmelon, the increase was also detected in the absence of chelator.

In most fruits the increase in pectin solubility that accompanies ripening has been related to endo-galacturonase activity (Pressey 1977, Huber 1983). The increase in pectin solubility may result from the hydrolysis of large pectin polymers as is the case with tomatoes (Gross and Wallner 1979, Huber 1983a, Rushing and Huber 1985) and peaches (Pressey and Avants 1978), or by cleavage of linkages between pectins and other cell wall components without further degradation as in apples (Knee and Bartley 1981). In muskmelon the increase in pectin solubility during ripening was accompanied by a decrease in molecular size of pectins (Figs. 12 and 13). This apparent degradation of pectin suggested that D-galacturonase may be active in muskmelon mesocarp. However, D-galacturonase activity was not detected in NaCl-soluble extracts or cell wall prepared from muskmelon mesocarp. Possible enzyme inactivation during extraction was ruled out based on results of experiments in which muskmelon and tomato pericarp were cohomogenized; D-galacturonase in the cohomogenates was 100% of the activity of equal quantities of tomato tissue alone. The lack of D-galacturonase activity in muskmelon is consistent with previous reports (Hobson 1962, Lester and Dunlap 1985). Strawberry fruit also lack D-galacturonase activity (Huber 1984). Although

there is a large increase in the quantity of soluble pectins during strawberry ripening there is no degradation of pectins. The locular gel of tomato fruit lacks D-galacturonase activity but there is an increase in the quantity of soluble pectins during development of this tissue (Huber and Lee 1986). Apples also have increased quantities of soluble pectins during ripening, but this occurs in the absence of D-galacturonase of the endo-type; however, apples do contain D-galacturonase of the exo-type (Knee and Bartley 1981).

In addition to modification of the pectic polysaccharides during softening, modification of the hemicellulosic component of cell walls has been detected in tomato (Huber 1983, 1986), strawberry (Huber 1984), and hot pepper (Gross et al. 1986). In muskmelon there was also a modification of hemicelluloses during fruit softening. Hemicelluloses from preripe muskmelon mesocarp were predominantly large molecular weight polymers, but as ripening proceeded there was a decrease in the molecular size of hemicelluloses (Fig. 14). Treatment of hemicellulose extracted from preripe fruit with protein extracted from ripe fruit showed a decrease in void material and an increase in included material similar to the transition from preripe to ripe *in situ* (Fig. 15). This indicates that hemicellulose modification may be mediated enzymically.

Compositional analyses of the hemicellulosic neutral sugars revealed changes in nonfractionated extracts as well as differential changes in the neutral sugar composition of large and small polymers (Table 4). These changes are evidence that cell wall modifications other than polyuronide degradation occur during muskmelon fruit softening. The decrease in galactose content from nonfractionated extracts was reflected in the loss of galactose from large polymers whereas small polymers increased in galactose content. The removal of galactose units from large polymers may account for the loss of galactose as well as the generation of small polymers. The results of hemicellulose compositional analyses differ from those reported for strawberry (Huber 1984) in which compositional changes did not take place along with the shift in polymer size.

The composition of total noncellulosic neutral sugar of ethanol powders also changed during muskmelon fruit softening (Table 5). A net loss of neutral sugars took place as ripening proceeded. Glucose, galactose, and xylose were the predominant neutral sugars present. A substantial (65%) loss of galactose occurred as muskmelon fruit ripened. Gross and Sams (1984) reported that galactose was the predominant neutral sugar in muskmelon cell wall and that it was the principle neutral sugar lost during ripening. In the present investigation composition

of ethanol-insoluble powders rather than cell wall was determined and this may account for some of the differences with Gross and Sams (1984) data. The large amount of glucose was apparently not due to the presence of starch as no starch could be detected in ethanol-powders using two different assay procedures.

The results of these studies indicate that the pectin and hemicellulosic fractions of muskmelon mesocarp cell wall are modified during the fruit softening process. The muskmelon fruit is unique in that pectin molecular weight decreases but in the absence of D-galacturonase activity. A change in pectin molecular weight in the absence of D-galacturonase has not been reported for other fruit. Hemicellulose metabolism also takes place during muskmelon softening and may be related to the increase in soluble pectin. Pectin solubility may be related to the cleavage of linkages between the pectins and hemicelluloses.

CHAPTER V
SUMMARY

The two principal components of muskmelon fruit quality are soluble solids content and flesh firmness. Although numerous studies have been conducted to evaluate the effects of various pre- and postharvest treatments on these quality parameters, physiological studies have been limited. Work presented in this dissertation addressed the accumulation of soluble sugar during muskmelon fruit development and the activity of invertase and sucrose synthase, two enzymes of sucrose metabolism. In addition, studies were conducted to determine the nature of changes in cell wall components in relation to fruit softening.

Although stachyose and raffinose are the two principal transport sugars in cucurbits and were detected in ethanol-soluble extracts prepared from muskmelon leaves and peduncles, these sugars were not detected in the fruit. Glucose and fructose in near equal concentration were the predominant ethanol-soluble sugars in muskmelon mesocarp during the early stages of fruit development. Sucrose accumulated rapidly during the latter stages of fruit

development and was the predominant sugar present in the ripe mesocarp.

A soluble acid invertase (pH optimum 4.0-5.0) was present in mesocarp extracts. Insoluble acid invertase accounted for only a small percentage (<1%) of the total activity. The apparent K_m for sucrose was 4 mM. Invertase activity was highest in young fruit and very low in ripe fruit. Sucrose synthase was also detected in the mesocarp extracts. The optimum pH for sucrose synthesis was 8.0. Activity of sucrose synthase was much lower than invertase. Sucrose synthase activity increased during fruit development. Invertase apparently prevents sucrose from accumulating during early fruit development, but as activity decreases, sucrose begins to accumulate. In the later stages of fruit development sucrose synthase may be responsible for the hydrolysis of sucrose.

Significant changes in cell wall components were detected during muskmelon fruit softening. There was a net loss of noncellulosic neutral sugar during softening and glucose and galactose accounted for most of the decrease. The glucose was apparently not the product of starch as no starch could be detected in ethanol-insoluble powders prepared from muskmelon mesocarp.

Total pectins in ethanol-insoluble powders and cell wall decreased slightly during fruit softening; however, there was a significant increase in the quantity of soluble

pectins during the transition from preripe to ripe. Gel chromatography of soluble pectins revealed a transition from predominantly large polymers in preripe fruit to smaller polymers in ripe and overripe fruit. The degradation of pectins was not the result of polygalacturonase activity. Polygalacturonase was not detected in salt-soluble extracts prepared from muskmelon mesocarp. The possibility of polygalacturonase inhibition or inactivation during extraction was ruled out based on results of cohomogenization experiments with tomato pericarp.

The hemicellulose (soluble in 4N KOH) fraction was also modified during fruit softening. Hemicelluloses from preripe fruit contained predominantly large molecular weight polymers; there was a decrease in the quantity of large polymers and an increase in smaller polymers as ripening proceeded. Changes in hemicellulose composition accompanied the changes in polymer size. Glucose and galactose were the predominant neutral sugars in the hemicellulose fraction. There was a net loss of these two sugars from the total hemicellulose fraction and from the large hemicellulose polymers. The modification of hemicelluloses appears to be enzymically mediated.

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He is happily married to Darrae Norling and the father of a son, Eric.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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